

STUDIES ON THE SHERRY FLOR

J. C. M. FORNACHON

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BY

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FOREWORD

CONTINUING the research work undertaken by the Australian Wine Board in collaboration with the University of Adelaide, which culminated in the publication in 1943 of "Bacterial Spoilage of Fortified Wine" by the Board's Research Officer, Mr. J. C. M. Fornachon, that gentleman was commissioned to conduct a research into the subject of "Flor" yeasts in relation to the production of Sherry.

This research later required finance in excess of that available from the Australian Wine Board, and in 1947 the assistance of the Commonwealth Scientific and Industrial Research Organization was sought. This assistance was readily granted, and the Wine Board and the C.S.I.R.O. now share the cost.

The University of Adelaide continued to grant very generous assistance in providing a laboratory at the Waite Institute for the conduct of the research.

Mr. Fornachon has devoted many years of close study covering the isolation of many strains of "Flor" yeasts, their individual reaction in different wines, and in wines of identical composition under various experimental conditions.

He does not claim to have completed this important research, but he believes he has reached a stage at which he could publish his findings so that they could provide a valuable guide to the future winemaker in the important field of sherry production.

He has produced regular half-yearly reports covering the progress of his studies, which have been made available to winemakers. Many of them have already applied the information they contained to improve the quality of their sherries, with the result that there are already considerable quantities of really high class "Flor" sherry produced in Australia, insufficient as yet to develop overseas markets, but it is confidently expected that production will slowly increase in course of time.

The Australian Wine Board, in publishing this book, wishes to thank the members of the scientific staff of the Adelaide University, especially Professor Prescott, Director of the Waite Institute, and Professor Cleland, the Chairman and members of the C.S.I.R.O., and the many winemakers who have collaborated with Mr. Fornachon in his research programme.

Mr. Fornachon has established himself as a scientist of very high standing, and we congratulate him upon his success in this important aspect of the development of the Australian wine industry.

KENNETH T. HARDY,
Chairman,
Australian Wine Board.

6th October, 1952.

PREFACE

SINCE Mr. F. de Castella introduced cultures of flor yeasts from Spain to this country in 1908 many winemakers have used the flor for making sherry of the 'fino' type. Although the use of these yeasts has led to a number of disappointments, particularly in the early years, it has been crowned with success on many occasions and Australian flor sherry was first marketed on a small scale round about 1930.

In 1940, because of the importance of sherry in the Australian wine trade, and because of the confusion existing regarding the nature of the flor organism and the way it should be used, the Australian Wine Industry, through the Australian Wine Board, asked the Oenological Research Committee to carry out investigations into the production of flor sherry with the object of making recommendations for the improvement of this type of wine. As a result of a survey of the position, made at that time, it was found that many of our leading winemakers had made or were making flor sherry in small quantities. Most of them had had some measure of success, in that they had produced some wines of high quality, but in general the results were much less regular and uniform than was desirable. As regards the actual flor process, the chief problems confronting winemakers seemed to be:

- (a) Failure of the flor film to grow or to persist on wine.
- (b) Lack of development of flor character even though film growth appeared to be satisfactory.
- (c) Occasional spoilage of wine by bacteria.

Of these mishaps, the first two were the most common causes of failures and the second was particularly puzzling to many winemakers, for it seemed logical to them that a vigorous growth of the flor film should always impart abundant flor character to the wine.

From a consideration of the information obtained in this survey, it was decided that the proposed sherry investiga-

tions should be concerned chiefly with the flor yeasts. In general terms, the objectives of the investigations were to determine what the flor organism is, what it does to wine and the effects of various factors on its growth and the changes which it brings about in wines. The work was commenced in 1941 and has been centred at the Waite Agricultural Research Institute near Adelaide. It has, however, included experiments carried out under industrial conditions in several commercial wineries in addition to laboratory experiments.

Much of the information resulting from the investigations has already been made available to the winemakers of Australia in the form of half-yearly progress reports, and many of the recommendations arising from the researches have already been widely applied with satisfactory results. Although there is still much to be learnt about sherry and the flor yeasts, and there is still ample scope for research in this field, it is considered that sufficient information has now been accumulated to justify the publication of the results herein reported.

During the course of the investigations a number of papers have been published by overseas workers in this field. Some of these papers, notably those of W. V. Cruess and his associates at the University of California, are already well known to many Australian winemakers, but others, particularly those from Spain and from Russia, are much less widely known and their contents have been briefly reviewed in this publication.

The writer wishes to acknowledge his indebtedness to Professor J. A. Prescott, Director of the Waite Institute, for his interest and advice during the course of the investigations, to Mr. K. P. Phillips for taking the photographs, and to Miss N. H. Bressler for the line drawings. Acknowledgement is also made to the many winemakers whose co-operation made it possible to extend the investigations from the laboratory to industrial wineries.

J. C. M. FORNACHON

August, 1952.

PREFACE TO THE SECOND EDITION

MR. J. C. M. FORNACHON wrote two books, "Bacterial spoilage in fortified wines" and "Studies on the sherry flor", both of which were published by the Australian Wine Board. These books have become standard reference works of very great value to the Australian wine industry, and have enjoyed a continuing demand over the years since their original publication. The first edition of "Bacterial spoilage in fortified wines" was exhausted some years ago and has since been reprinted.

This book, "Studies on the sherry flor", has also been the subject of such interest as now to necessitate this second edition. It shows how well Mr. Fornachon carried out the original research reported in these books that both are still relevant; in fact, in considering what amendments would be desirable in the light of modern developments, it was thought most suitable to reprint the original text, adding the minimum amount of additional bibliography to indicate to the reader the direction of later research. This additional material is included as a short section, "Later Research", at the end of the book. The book may still be read with great profit as a reference for the behaviour of the sherry flor by the winemaker and student alike.

Mr. Fornachon carried out the original research reported in this book as Officer-in-Charge of the C.S.I.R.O. Unit of Oenological Investigations and Senior Research Officer of the Australian Wine Board. When the Australian Wine Research Institute was founded to assume responsibility for oenological research, Mr. Fornachon was appointed foundation Director of Research. Until his untimely death in 1968, he continued active research in the field of microbiology of wine, an area in which he had achieved an international reputation, and published a series of important scientific papers.

W. W. FORREST,
Director of Research,
The Australian Wine Research Institute.

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CHAPTER I

INTRODUCTION

THERE seems little doubt that the name 'Sherry' is derived from that of the town of Jerez de la Frontera in the centre of the area producing this type of wine in Western Andalusia in the South of Spain. There is not now any very obvious connection between the spelling or the pronunciation of Sherry and Jerez, but both have undergone some modifications through the years which have elapsed since wines from Jerez were first shipped to England. According to a recent article in the 'Bulletin de l'Office International du Vin' (1950), many historians believe that the town of Jerez, formerly spelt Xérès, is the same as the ancient Roman town of Ceret (or Serit). During the Moorish occupation of the country, the name was pronounced Scheres or Scheris by corruption of the Roman word Ceret. In later years the spelling was changed to Jerez by the Spaniards who pronounce the word as 'Hereth', the initial 'J' in Spanish being pronounced rather like the English 'H'.

It is evident that the early Moorish pronunciation was the one brought to England, however, for the first form of the word in English was 'Sherris' used as an abbreviation for 'Sherris Sack.' Because 'Sherris' was mistakenly considered to be plural the word 'Sherry' was coined as the singular form, and has since come into general use to denote the wines of Jerez de la Frontera and other wines of similar type.

Historical records indicate that vines have been grown and wines made in the Jerez region from very early times and that some of these wines were exported to England at least as early as the twelfth century. The real trade in sherry came later, however. According to Allen (1931), the development of the wine trade between Jerez and England followed the agreement of 1517, whereby the Duke of Medina Sidonia granted special concessions to English

merchants to ship the wines from this region. These wines were called Sack or Seck (from the Spanish 'Seco'=dry) because, although they were not dry by present-day standards they were drier than those imported from Spain previously.

The Sack from Jerez, or 'Sherris Sack' as it was called, became popular in England during the sixteenth century and gradually replaced wines of the Canary Islands previously in favour. The popularity of sherry continued during Elizabethan times and during the wars with Spain, supplies are said to have been maintained by cargoes captured at sea. Drake is reputed to have captured 2,900 butts of sherry in a raid on Cadiz in 1587. The vogue of sherry in England at this time is indicated by the numerous references to it in Shakespeare's plays, such as the much quoted passage in 'King Henry IV' where Falstaff sings of 'good Sherris Sack' and its 'twofold operation'.

The popularity of this wine in England continued during the seventeenth century but later declined. It returned in favour under the modern name of 'Sherry' towards the end of the eighteenth century, however, and its popularity continued, reaching its zenith in 1873 when over eight million gallons of sherry were exported to England. The trade then gradually declined to about one million gallons by 1919, and subsequently increased again to over three million gallons in the late 1930's, according to statistics published by the Office International du Vin of white wine exported from Spain to the United Kingdom. More recent figures from this source show further fluctuations during the last decade and the most recent figure available—that for 1951—was 156,567 hectolitres or nearly three and a half million gallons.

Not only has the volume of the English sherry trade fluctuated widely, but the style of the wine exported from Jerez to England has changed very considerably down through the years. The Sherris Sack of Shakespearean times, which was lauded by Falstaff, was a dark-coloured rather bitter wine probably similar to *Vino de Color* or *Macetilla* of the present day. This is made by boiling down part of the must and adding this 'Arrope', as it is called, to the remainder before or during fermentation which pro-

ceeds slowly and eventually yields a very aromatic wine, more or less sweet, distinctly bitter, and with a curious cooked, almost burned flavour. Such wine is now used only for blending and imparting colour to brown sherries.

The early sherry gradually gave place to more natural types of wine, almost free of boiled must though slightly—and often distinctly sweet, typified by the after-dinner Golden Brown and Old East India sherries of last century. Incidentally, the name East India sherry had nothing to do with the country of origin of these wines, which was of course Spain and not India. The name was derived from the common practice of sending casks of wine on a long sea voyage to the East Indies and back in the hold of a ship in order that the ageing of the wine might be advanced by the continuous movement of the ship. This effect of a sea voyage on the ageing of wines was known to the Romans and was referred to by Pliny in the first century A.D.

Since the days of the East India sherries another change has come about in the style of the wines of Jerez in favour of the paler, drier types. This change was due to the discovery, during the latter half of the last century, of the beneficial rôle played by a film-forming micro-organism which grew on the surface of wines exposed to the air in Jerez and was known locally as 'la flor' (the flower), for the development of this film was referred to by the local wine-makers as 'the flowering of the wine'. The presence of the flor must have often been noticed on the wines of Jerez when casks were not filled and tightly bunged, but its beneficial effects on the wine do not seem to have been appreciated until about 1850. Before then, wine which had flowered heavily was considered to have been spoiled. Even for some time after this date the nor wines were not appreciated by many sherry shippers or consumers, but gradually, as more was learned of how to use the nor, these wines came into favour and the fino sherries had arrived. The present-day fino sherries are pale-straw to light-amber in colour and dry or nearly dry. There is also, of course, a demand for other types of sherry—the full bodied sherries which are darker in colour and usually slightly or distinctly sweet—but the

fino sherries are today the most popular type and generally represent the highest quality and the highest priced of these wines.

Thus, in the way outlined above, the present-day sherry, or more correctly the present-day sherries (for there are many different styles of them) have evolved. They are essentially blended wines. The terms 'vintage year' and 'vintage wine' which are applied to many wines and which are used to denote a specially favourable year or a high quality wine produced in a particular year, find no such application in speaking of sherry. Although it is true, of course, that better wine is made in some years than in others, the particular system used for maturing sherries in Spain ensures that the wine made in any particular year is not kept separate but loses its identity in order to contribute to the quality of the whole blend. Morton Shand (1929), referring to the technique of blending in Spain, claims that, 'the secret of fine sherry is the secret of skilful blending'. This statement, of course, presupposes that wines of suitable types and of suitably high quality are available for blending. Actually a large number of different styles of sherry are exported from Spain and these are obtained by skilful blending of a comparatively few basic types which may be broadly grouped as 'fino', 'amontillado' and 'oloroso'.

The fino group comprises those wines which have been covered by the flor film during their maturation and have thus acquired the distinctive flor character. They are pale straw-coloured wines, dry or almost dry on the palate and with a pronounced and characteristic bouquet and flavour of which acetaldehyde is an important component.

The amontillado wines are essentially a sub-type of fino and have been matured under the flor film during their early stages, but, with continued ageing after the disappearance of the film, they have become rather darker in colour, lost their fresh flor character, taken on a characteristic nutty flavour, and increased somewhat in alcoholic strength. The amontillado represents a later stage of development of the fino.

The oloroso wines are more full-bodied and darker in colour than the finos. This group includes wines which started out as finos but which became too coarse to be retained in that category and have accordingly been reclassified.

Within each of the broad groups of wines there are various sub-types, and there are also subsidiary or auxiliary wines used for imparting colour or sweetness to the basal wines by blending. It is not intended to discuss in detail here the systems of production, classification and blending used in Spain. For such details the reader is referred to the writings of Castella (1909, 1926), Cruess (1937, 1948) and Allen (1931, 1933).

In view of the widespread popularity of sherry, it is not surprising that other winemaking countries, with climate similar to that of Jerez, should endeavour to make wines of this type. It must be confessed that the results of many of the early attempts bore only a faint resemblance to the wines of Jerez, but, as more has been learned about choice of suitable varieties of grapes, methods of making the wine, and particularly, the use of flor yeasts in the maturation of the wine, the results have been more promising. It is now quite true that high quality wines of sherry types are produced in several countries other than Spain—notably Australia, South Africa, California and Portugal. In fact, there are numerous authentic instances when experienced judges have pronounced such wines to be high quality Spanish sherries in 'blind tastings'.

As with all wines, the quality of sherry depends on the interaction of a number of factors. It depends, of course, on the quality of the raw material, that is, the grapes, which in turn depends on many factors, of which the most important are the variety, the stage of ripeness, the climate under which they have been grown and the weather during the current season, the soil on which the vines are grown, the systems of pruning, cultivation and manuring, and the presence or absence of vine diseases. Quality of wine is also determined in large measure, however, by the way in which the raw material is processed; by the methods used and the care

with which the methods are applied to vinification, maturation, and handling of the wine. Sherry, then, owes its particular characteristics and its quality to all these factors, but it owes much, perhaps more than any other wine, to the methods and care of making and maturing.

If, in the following pages, discussion is centred round the making and maturing of sherry with relatively little discussion of the factors determining the quality of the grapes, it should not be inferred that these latter are considered to be of minor importance. On the contrary, they are of major importance and no amount of care in making and maturing the wine can produce really high quality sherry from unsuitable or inferior grapes.

CHAPTER II

THE NATURE OF THE FLOR ORGANISM

THE appearance of the flor film on the wines of Jerez de la Frontera has attracted the attention of many writers, including the authors of both technical and popular accounts of sherry making, and it must be admitted that some of the published accounts of the flor film and the flor organism are far from accurate. Until recently, there has been much confusion in the literature as regards the identity of the flor yeasts. The Spanish word 'flor' means 'flower' and was applied to this film because the ancients referred to the growth of this film as the 'flowering of the wine'. The equivalent of this term has been used in many countries to indicate the growth of film-forming yeasts, usually *Mycoderma vini*, on the surface of wines of low alcohol content which have been left exposed to the air. Thus in English-speaking countries the film formed on wine by *Mycoderma vini* is commonly known as 'flowers of wine', while the French refer to it as 'la fleur'. Because of some resemblance between the appearance of the flor film and that of ordinary flowers of wine, many writers seem to have assumed that they are both formed by the same organism, that is to say, by *Mycoderma vini*, but in reality the similarity in appearance is only very slight.

One of the first writers to publish a detailed description of the making of sherry was Roques (1903) who referred to the flor as *Mycoderma vini*, simply because of the slight similarity, already mentioned, between the films. This name was then applied to the sherry flor organism by the majority of writers of both technical and popular articles on sherry during the next thirty years, although it was, of course, realized that the flor film of Spain did not affect the wine in the same way as did 'la fleur' or flowers of wine formed by *Mycoderma vini* in other countries. Whereas

the flor film imparts the special sherry flavour and aroma to wine, the flowers of wine or *Mycoderma vini* film simply brings about a flatness or loss of flavour and aroma. To explain such differences, some writers have claimed that a special strain of *Mycoderma vini* was responsible for the flor film, and others, that some special circumstance associated with the wines of Jerez caused the *Mycoderma vini* to affect these wines in a special way quite different from that usual in other countries.

Some writers who have applied the term *Mycoderma vini* to the flor, have quoted in support of this usage, Pasteur's account (1864), of the maturation of the 'vin jaune' or yellow wine of the Jura, in which he discusses the rôle of *Mycoderma vini*. It is commonly conceded that these wines bear some resemblance to sherry, but it should be remembered that the term *Mycoderma vini*, as used by Pasteur, had a very different significance from what it has to-day. The term *Mycoderma* now means a particular genus of non-fermenting, film-forming yeasts with oval cells which multiply by budding, do not form spores and have certain other well-defined characteristics. As used by Pasteur, however, the term meant little more than 'any micro-organism which formed a film on liquid media'. Although Pasteur used the term in a generic form, little was then known about classification of micro-organisms and modern methods of pure culture had not then been developed. In the past, many different types of yeasts and even bacteria have been labelled as *Mycoderma*. For instance, Pasteur discusses *Mycoderma aceti* (a bacterium) and *Mycoderma vini* (a yeast) as though they belonged to one genus. Of course, Pasteur was well aware of the wide differences between these two organisms, but it is obvious now that such widely different organisms cannot be included in one genus. The name *Mycoderma* has, therefore, been retained for a particular group of film-forming yeasts as indicated above, and other film-forming organisms have been classified and given other names.

Reference to Pasteur's own account of the film-forming yeasts occurring on the Jura wines and which he called

Mycoderma vini, makes it obvious that he was dealing with more than one organism under this name. The organism depicted by Pasteur in Fig. 3 of his paper, and which he claims is necessary for the development of the characteristic bouquet of these wines has broad oval to rounded cells in contrast to the long oval cells which he depicts in Fig. 2 and which are more typical of *Mycoderma vini*. The yeast with the rounded cells might well belong to the genus *Saccharomyces*, but the description given by Pasteur is not sufficiently detailed to allow identification. Thus we see that Pasteur's use of the name *Mycoderma vini* in discussing the film yeasts of the Jura, is no justification for modern use of the name for the flor yeasts of Jerez.

Although winemakers and oenologists are more familiar with the film-forming habits of *Mycoderma vini* than those of other yeasts, the ability to grow as a film on liquids is not confined to this species by any means, but is common to many different yeasts which have an oxidative metabolism or which require free oxygen for their development. Broadly speaking, yeasts fall into three categories regarding their ability to form films on liquid media containing sugar, such as grape juice or beer wort.

(a) First, we have those which grow as a film from the start in such media. This group includes the well-known *Mycoderma vini* and also many other yeasts, some of which, such as *Pichia*, are important to the winemaker, usually as agents of spoilage. Yeasts belonging to this group are poor fermenters, forming only small amounts of alcohol and many of them do not cause any visible fermentation at all.

(b) Then we have yeasts which do not form a film at all in such liquids, but grow as a deposit and may, or may not, bring about an alcoholic fermentation. This group includes many, but not all, of the ordinary wine yeasts (*Saccharomyces ellipsoideus*), and also many other industrial yeasts.

(c) Finally, we have a third category which, when seeded in grape juice or beer wort, commence growing as a deposit and bring about an alcoholic fermentation and then later, when the sugar has been consumed and if conditions are favourable, grow on the surface of the liquid where they

oxidise alcohol and other constituents of the fermented liquid. The type of surface growth varies considerably according to the strain of yeast, the composition of the medium and other conditions. It may consist only of a ring round the walls of the tube or flask containing the culture, it may consist of islets on the surface of the medium, it may be a thin continuous veil over the whole surface, or it may be a more or less thick opaque film varying in colour from almost white through grey to brown, and in appearance, from waxy and almost smooth, to dry and heavily wrinkled. This group includes the vigorous film-forming yeasts known as sherry flor, but it also includes a great number of other yeasts including many common wine yeasts, though these latter usually do not have the film-forming character so strongly developed as the flor yeasts. The property of film formation is much commoner among wine yeasts than is commonly realized, in fact many winemakers are surprised to find wine yeasts forming films at all. Nevertheless, Hansen had shown as early as 1886 that some true *Saccharomyces* yeasts, including *Saccharomyces ellipsoideus*, are able to grow as films on the surface of liquids and he defined the conditions necessary for the formation of films by these yeast as follows:

- (i) The primary fermentation must be terminated. That is to say, the fermentable sugar must have been used up.
- (ii) The surface of the liquid must be undisturbed.
- (iii) There must be free access of air.
- (iv) The temperature must be suitable. Hansen actually determined the temperature limits for film formation by several species of *Saccharomyces* yeasts and found these limits to be narrower than the temperature limits for growth as a deposit and fermentation by the same yeasts.

Some indication of the frequency of the film-forming habit among wine yeasts is given by the work of Schanderl (1936), who cultivated 138 races of yeasts from the Geisenheim collection in grape juice diluted to 70° oechsle (9.4°B°) in flasks closed with cotton bungs and incubated at 25° C. It was found that after the fermentation was completed 23 yeasts (16.5 per cent.) formed strong films within four

months, 62 yeasts (45 per cent.) formed a thin veil or islets on the surface, 34 yeasts (25 per cent.) formed a ring at the surface and only 19 yeasts (13·5 per cent.) did not form any surface growth at all. Schanderl further showed that the film stage of growth usually had a lower alcohol tolerance than the fermentative stage of the same yeasts. Thus to Hansen's four conditions necessary for film formation by the *Saccharomyces* may be added a fifth; i.e., the alcohol content of the liquid must not be too high.

Some of these conditions, which are necessary for film formation, be it noted, are just what the maker of wines other than flor sherry, seeks to avoid. He does not allow dry wines of low alcohol content to remain exposed to the air, for he knows that these conditions favour the development of the acetic acid or vinegar bacteria which would spoil his wine. For this reason he does not see the films which many of the yeasts he uses would be capable of forming, if they were allowed to do so.

During the last twenty years the sherry flor yeasts have been studied by investigators in many countries, including Spain, Russia, Germany, California, South Africa and Australia, and some of the investigators have worked with material obtained from Southern Spain. The publications of these workers (Marcilla 1939; Prostosserdov 1934, 1944; Prostosserdov and Afrikian 1933, Frolov-Bagreev and Saenko 1945; Schanderl 1936; Hohl and Cruess 1939; Cruess 1948; Niehaus 1937; Williams 1943) have made it abundantly clear that the predominating organisms in the flor film are yeasts which belong to the genus *Saccharomyces*. Like other *Saccharomyces*, these flor yeasts multiply by budding but form spores under appropriate conditions and they bring about a normal alcoholic fermentation when seeded in grape juice. Once this fermentation is completed and if the fermented liquid is kept at a suitable temperature (between about 10° and 25° C.) and exposed to the air, the yeast grows on the surface as a grey to cream-coloured film which, under favourable conditions, gradually becomes thick and much wrinkled (see plates). As far as film formation is concerned then, the sherry flor yeasts differ from

most other wine yeasts in having a much more strongly developed film stage of growth. They form thicker and more stable films and the film stage has a high tolerance of alcohol—varying from about 14.5 to 17 per cent. by volume, depending on the strain of yeast, the temperature and other factors.

The first to show that the flor yeast was capable of fermenting grape juice and belonged to the genus *Saccharomyces*, seems to have been Frolov-Bagreev who, according to Frolov-Bagreev and Saenko (1945) established this fact in 1908. However, by far the most complete description of the sherry flor yeasts is that of Marcilla and his associates (1939), who named their organism *Saccharomyces beticus* and recognized three races or varieties (α , β and γ) of this species. Mrak and Phaff (1948), however, consider that Marcilla's race γ might more fittingly be considered as a variety of *Saccharomyces chevalieri*.

Descriptions published by other workers indicate that some film yeasts which impart a flor character to wines, differ in their fermentation reactions and cultural characters from the yeasts described by Marcilla, and so belong to other species of the genus *Saccharomyces*. Thus Prostosserdov (1944) claims that some flor yeasts obtained from Spain and studied in Russia, were *Saccharomyces heterogenicus*, while strains isolated in Armenia proved to be *Saccharomyces oviformis*. Previously, Prostosserdov (1933) had proposed the name *Saccharomyces cheresiensis* for the sherry flor yeasts, a proposal which was criticized by Schanderl (1936), who rightly pointed out that the ability to grow as a film after fermentation was completed, was not in itself a distinctive enough character to warrant setting up a new species of the genus *Saccharomyces*. However, Schanderl does not appear to have examined sufficiently the other characteristics of the yeast which he studied to determine whether different species were involved or not. The flor yeasts studied by Hohl and Cruess (1939) seem to have included representatives of several species of *Saccharomyces* including some very similar to *Saccharomyces beticus* (Marcilla). Scheffer and

Mrak (1950) refer to strains of sherry flor yeast isolated from California wines as *Saccharomyces chevalieri*.

This seeming confusion of species names is resolved if we remember that species in the genus *Saccharomyces* as classified by Stelling-Dekker (1931), are differentiated on the basis of their ability to ferment various sugars and their morphology.

As previously pointed out, the property of fermenting grape juice and then growing as a film on the resulting wine is not confined to any one species of the genus *Saccharomyces*, but is common to several, and it seems that there are strains or varieties of yeasts belonging to several species which have the film stage of growth sufficiently strongly developed to behave as flor yeasts. Actually, it has been found that this property of vigorous film development is not a constant property of a particular strain of yeast but may be lost under certain conditions even although the other properties of the yeast seem to be unchanged.

It should be noted in passing that the occurrence of sherry flor yeasts is not confined to Spain. Yeasts of similar type have been isolated from local sources in the Soviet Union and in South Africa, and the writer has also isolated them from the surface of grapes in Australia, while Hohl and Cruess isolated them from material obtained from Arbois in France as well as from Spain.

The possibility of yeasts other than *Saccharomyces* playing an important and useful rôle in the production of sherry has been raised from time to time, but up to the present there does not seem to be sufficient evidence available to pronounce judgment. De Castella (1909) refers to claims made to him in Spain, for the beneficial effects of certain apiculate yeasts growing in the must during the early stages of the primary fermentations. Bobadilla (1943) also makes this claim.

Film-forming yeasts other than *Saccharomyces* have been isolated from flor films by several workers. Thus Hohl and Cruess (1939) isolated several yeasts belonging to the genera *Pichia* and *Hansenula* from cultures of sherry yeasts and suggested that since yeasts belonging to these groups

are notoriously prolific as producers of volatile esters they might have a desirable effect on the aroma and flavour of the wine. These workers were not able to give any estimate of whether such yeasts were actually present in significant numbers in the original flor films from which their cultures were derived, and since the yeasts failed to grow on wines containing 14 per cent. of alcohol in laboratory trials, their rôle in the maturation of sherry seems doubtful. Marcilla (1939) also mentions that strains of *Pichia* have occasionally been isolated from flor films on wines in Southern Spain, but only from wines in which the alcohol content had fallen considerably owing to evaporation or other causes. In most of the wines he examined the film consisted of *Saccharomyces beticus* in almost pure culture.

Some interesting claims of the use of yeasts other than *Saccharomyces* in making sherry have come from Russia. Sherbakov (1941) reported that sherry character was imparted to wine during the primary fermentation when grape juice was fermented with a particular fission yeast which was isolated from wine near Moscow in 1934 and called *Schizosaccharomyces moscowia*. On the other hand Frolov-Bagreev and Saenko (1945) isolated non-fermenting film-forming yeasts belong to the genus *Hansenula* from a sample of flor obtained from Spain. Since these *Hansenula* yeasts failed to grow on wine of normal alcohol content, but readily form a film on grape juice, the Russian workers adopted the procedure of growing them on the grape juice for three days before seeding with the fermentative flor yeast. In other experiments the must was seeded simultaneously with the two types of yeast mixed in various proportions. It was claimed that the use of *Hansenula* yeasts in this way gave increased production of acetal and volatile esters and hastened the development of sherry aroma in the wine. These claims open up some interesting possibilities, but although the presence of various yeasts other than *Saccharomyces* during the making of many wines, is well established, claims for their beneficial effects are less well founded, and practical methods of using them have yet to be demonstrated. The use of *Pichia* and *Hansenula* yeasts in conjunction with the

flor, in this laboratory, has not improved the quality of the product.

In this publication, only those yeasts which are capable of fermenting grape juice and of subsequently growing as a film on the wine are referred to as flor yeasts. Although, as indicated above, the literature indicates that at least some strains belonging to several species of yeasts fulfil these conditions, it will be shown that those studied in this country belong to the species *Saccharomyces beticus* (Marcilla).

CHAPTER III

THE FLOR YEASTS IN USE IN AUSTRALIA

IN order to establish the identity of the flor yeasts used in this country, and to compare them with those used in Spain and in other countries, the characteristics of these yeasts have been determined by detailed laboratory studies. The information thus obtained has been compared with descriptions of flor yeasts published by other workers, and the writer was fortunate in obtaining cultures from the late Professor Marcilla of Madrid and Professor W. V. Cruess of California, so that direct comparisons with the yeasts described by these workers could be made.

The sherry flor yeasts in the Waite Institute collection and which have been studied during the course of these investigations, comprise 29 strains as follows:

2 strains of authentic Spanish origin obtained from Madrid and labelled *Saccharomyces beticus*.

2 strains of reputed Spanish origin kindly supplied by Professor W. V. Cruess, University of California.

1 strain which came from the Arbois district in France was also supplied by Professor Cruess.

20 strains of reputed Spanish origin, which were isolated by the writer, from flor films growing in Australian wineries which had used flor cultures said to have been introduced from Spain at various times.

4 strains which were isolated by the writer from the surface of grapes growing in South Australian vineyards. Regarding this group, it is worthy of note that although two of these strains were isolated from vineyards near wineries where flor cultures are used, the other two were isolated from grapes growing in the Fullarton collection, which is several miles from, and has no direct connection with, any winery.

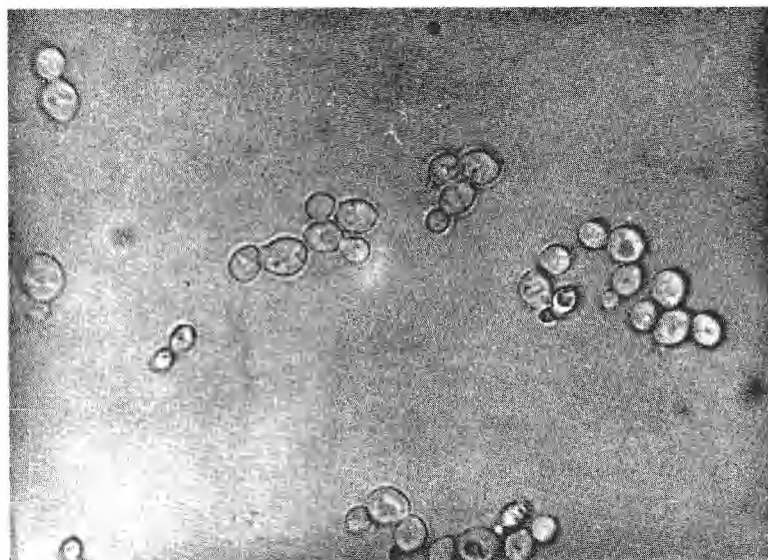


PLATE 1.

Flor yeast after 24 hours in grape juice. Unstained. $\times 900$.

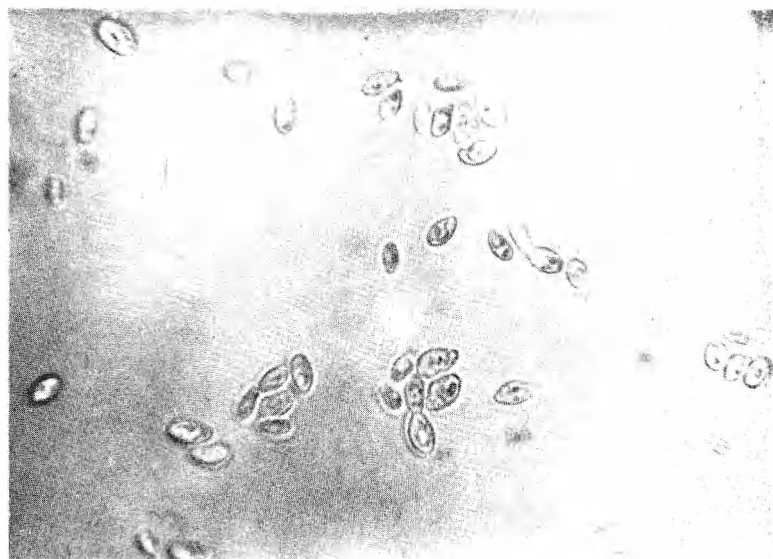


PLATE 2.

Flor yeast cells at the beginning of film formation on wine.

Unstained, $\times 900$.

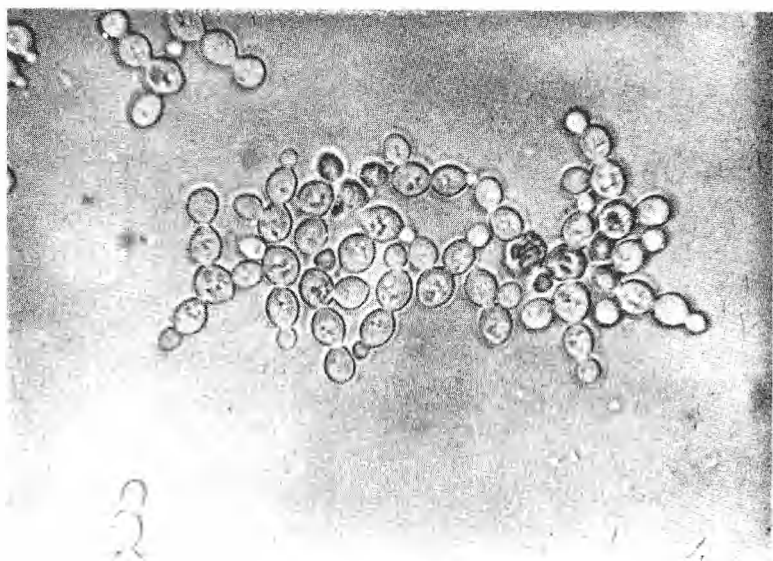


PLATE 3.

Flor yeast cells from 19-day-old film on wine.

Unstained, $\times 900$.

In addition to the foregoing strains of flor yeasts, several strains of yeasts belonging to the genera *Pichia*, *Hansenula*, *Torulopsis* and *Candida*, of reputed Spanish origin, were included in the collection. None of these, however, has yet proved capable of growing on wine containing more than 12 per cent. of alcohol by volume.

Before studying the flor yeasts it was, of course, first necessary to isolate them in pure culture. The actual procedure used for isolation differed according to the circumstances. Cultures which were received as pure cultures were simply streaked on the surface of diluted-grape-juice-agar medium (see Appendix) in petri dishes, incubated at 25° C., and the resulting colonies examined in order to verify the purity of the culture. Samples of flor received from wineries were grown as plate cultures as mentioned above, and were also seeded into flasks of sterile grape juice and incubated at 20° C. These flasks were allowed to ferment and were then left for a further period till flor films had developed and these were, in turn, plated out and typical colonies picked off and seeded into fresh medium. To isolate flor yeasts from grapes, the following method was used. Small twigs of grapes were clipped from the vine with sterile scissors and allowed to fall into sterile screw-capped jars. The grapes were then taken to the laboratory and added to small flasks of sterile diluted grape juice to which between 5 and 7 per cent. by volume of alcohol had been added. The flasks were incubated at 20° C. and kept at this temperature for six weeks after visible fermentation had ceased or until a film had formed. This film was then used to inoculate sterilized dry wine containing 14 per cent. by volume of alcohol, and from the film which grew on the wine plate cultures were inoculated for isolation.

For the purpose of identification, the morphological, cultural and biochemical characters of the various strains of yeasts were determined in laboratory cultures on several of the culture media commonly used for yeasts. Details of the preparation of these media are given in Appendix I.

On the basis of the tests carried out, the yeasts studied fall into three groups or types, as follows:

TYPE I

This comprises one strain received from Spain, two from California, fifteen of reputed Spanish origin obtained from Australian wineries, and four strains isolated from local grapes. This type has the following characteristics:

Growth in malt extract medium at 25° C.

At the end of 24 hours the liquid is slightly turbid and the cells are oval to broad oval, mostly 4 to $6\mu \times 5$ to 8μ , occurring singly, in pairs and sometimes in small clusters and short chains of three or four cells. Vegetative multiplication is by budding, usually at or near the ends of the cells.

After 48 hours the liquid is turbid, a deposit has formed and a very slight fermentation is evident.

After 72 hours the liquid is still turbid, but there is only very slight or no visible fermentation. The cells are mostly of similar dimensions to those seen at 24 hours, although a few longer cells (3 to $6\mu \times 6$ to 9μ) and giant cells ($8 \times 9\mu$) are also present.

After three to five days the liquid clears, leaving a powdery deposit, and there can be seen small granules of yeast cells adhering to the walls of the tube or flask, usually on one side, which on succeeding days increase and sometimes extend right to the surface of the liquid. These granules evidently correspond to the 'levadura ascendente' or climbing yeasts described by Marcilla (1939).

By the sixth to tenth day a white surface film has been formed, which soon thickens and in this medium falls very easily if disturbed. The cells in the young films on malt extract are round to broad oval (4 – $7\mu \times 5$ – 9μ) occurring singly and in pairs but very often in clusters and short chains, and many cells bud from two points simultaneously.

Growth in grape juice of 13 to 14°B° at 20° C.

At the end of 24 hours the liquid is turbid and fermentation is evident. The cells are similar to those seen in malt extract but slightly smaller (4.5 – $6\mu \times 5.5$ – 7μ). Fermenta-

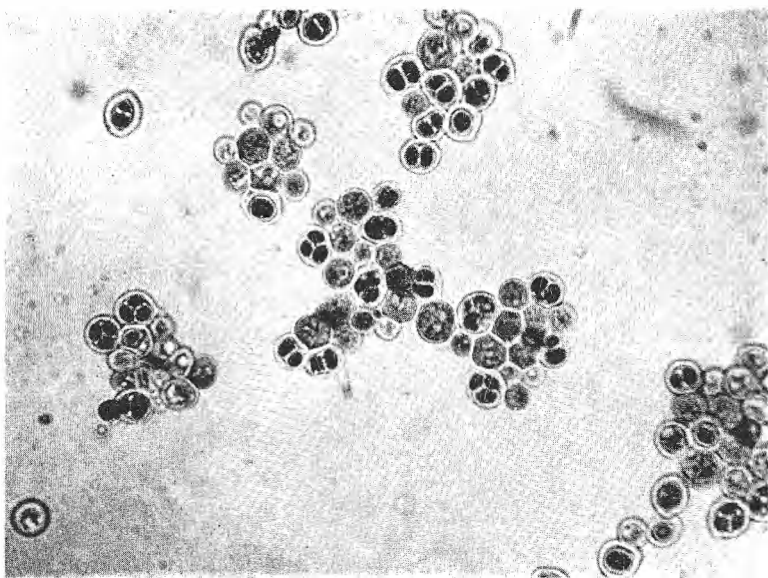


PLATE 4.

Flor yeast cells with spores. 12 days on plaster slant.
Stained with carbol-fuchsin and methylene blue. $\times 900$.

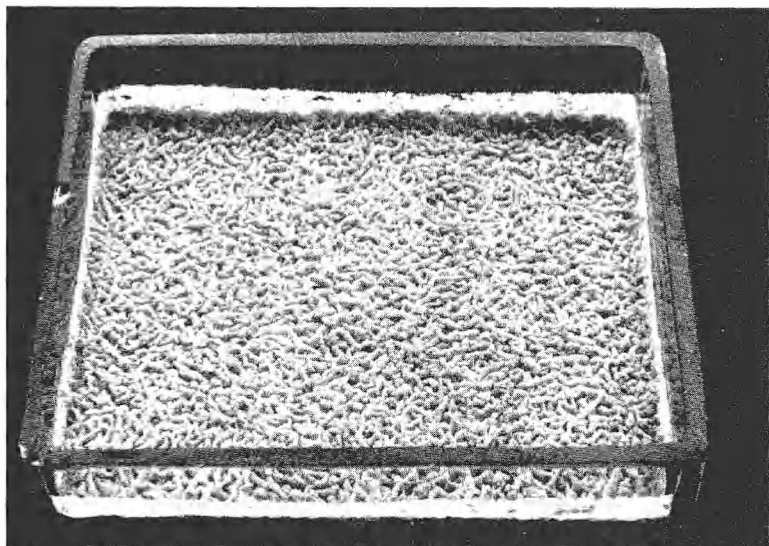


PLATE 5.

Fully developed floe growth on wine. Upper surface of film.

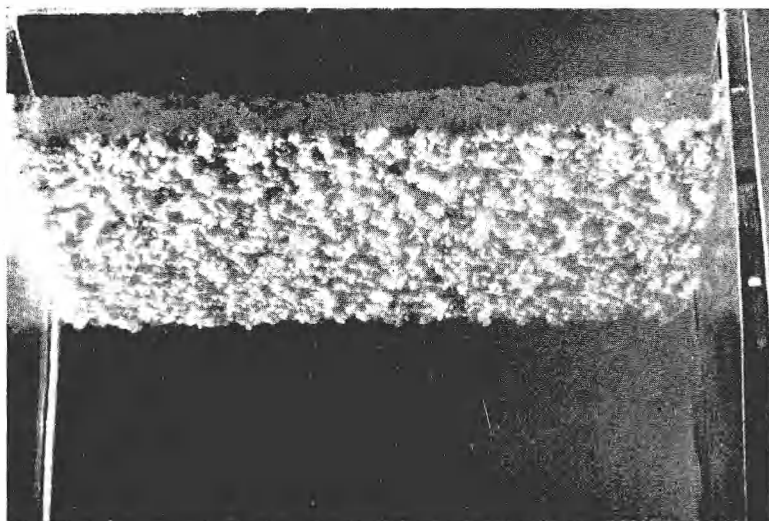


PLATE 6.

Fully developed floe growth on wine. Lower side of film.

tion becomes vigorous within two to three days and in laboratory cultures is complete in about ten days in test tubes or longer periods in flasks. When fermentation has ceased the liquid becomes clear and the granules of 'climbing yeasts' mentioned previously can be seen deposited on the walls of the vessel. Film formation commences as a surface ring and semi-transparent islets of thin membranous surface growth. Within a few days of their first appearance the islets become confluent to give a very thin semi-transparent veil with small islands of more opaque film—the fine lace stage of Marcilla. The film then gradually increases in thickness, becoming opaque and at first smooth and membranous, then creased and finally, if conditions are favourable, heavily wrinkled and convoluted at the surface. The colour, at first white or cream, may remain pale or become grey or even dark brown, depending on cultural conditions. The effect of various conditions on growth and appearance of the film will be discussed in greater detail later, but usually the film becomes dark when growing under unfavourable conditions or when very old.

Cells from a very young film, that is when it can just be detected, are usually smaller and more narrow when the fermenting cells (Plate 2), but by the time the young film has reached the 'fine lace' stage the cells have become larger and broader, resembling in shape those found in fermenting cultures. The cells in young films commonly bud from two or more points at a time and occur in clusters and in short chains (Plate 3).

Although the narrow cells shown in Plate 2 are found on the surface of wines in the very early stages of film development, they also occur in older films which are growing very slowly under adverse conditions.

Growth on malt extract agar slants at 15° C.

Cells in young cultures are similar to those in liquid malt extract medium. After six weeks, growth on this medium is raised, cream-coloured, and usually with a waxy lustre though somewhat moist and glistening in some strains. The surface is smooth and the margins are smooth to slightly lobed.

Giant colonies on malt extract gelatine at 15° C.

After six weeks, giant colonies are cream-coloured, round, 8 to 10 mm. in diameter, usually conical in elevation and often with a depressed centre. The appearance is usually dull and wax-like. The surface varies from almost smooth to much wrinkled, and the margins from slightly lobed to serrated. Actually no two strains of the flor yeasts studied produced identical giant colonies, although the differences between many of them were very slight. None of the strains studied liquefied gelatine.

It has been the writer's experience that the finer details in the appearance of giant colonies vary so greatly with the composition of the medium and other cultural conditions, that these characters are of very limited value in the taxonomic description of yeasts. Even when every effort is made to standardize technique and media, colonies of a single strain often differ slightly on two batches of malt extract gelatine. Giant colonies have often been found useful, however, in deciding whether two cultures of yeast are identical or not, provided that they are grown side by side in the same petri dish of medium. For example, when experiments are carried out in wineries which have previously used flor yeasts, it is often very important to know whether the films which grow in the casks really represent the strain seeded there experimentally. If samples of the films are collected from such casks and giant colonies are grown from them, side by side with giant colonies from a laboratory stock culture of the experimental strain and the winery strain, a careful comparison of the colonies will often reveal whether the experimental strain has been established in the winery or not.

Sporulation

The various strains studied differ considerably in their spore-forming ability. Several of them produce spores very abundantly, but the majority produce only a few spores and in four strains, which conform to this type or group in all other respects; spores have not been detected on any of the media used. In most strains spores are formed most

readily on plaster slants, but Gorodkowa agar, Mrak, Phaff and Douglas' vegetable extract agar and Adams' dextrose acetate agar give good results with some strains. Only a few strains form spores on carrot wedges.

Spores are formed without any sign of conjugation between cells. They are smooth, oval, 2.5 to $3\mu \times 3$ to 3.5μ , and number 1 to 4 per ascus (see Plate 4). Plaster slants were found to give the greatest proportion of 4-spored asci while 2-spored asci were most abundant on Gorodkowa agar.

Fermentation of sugars

In yeast extract medium glucose, fructose, mannose and sucrose are fermented, but not galactose, maltose, lactose or raffinose.

Utilization of carbon sources

In artificial medium growth occurs when any one of the following are supplied as sole source of carbon: glucose, fructose, mannose, sucrose, maltose, galactose, raffinose, glycerol, ethyl alcohol, acetic acid and lactic acid. Tartaric, malic and citric acids supported only weak growth when they were the only carbon sources present, but they stimulated growth appreciably in artificial medium containing ethyl alcohol and no other carbon source.

Utilization of nitrogen sources

In artificial medium, the yeast utilizes ammonium sulphate, asparagine, peptone and urea, but not nitrates.

TYPE II

This group comprises one strain received from Spain and three of reputed Spanish origin obtained from Australian wineries. These yeasts differ from those of the first group in their vigorous and regular fermentation of maltose. Only the Spanish strain in this group had the film-forming character sufficiently strongly developed to form a vigorous film regularly on wines containing 14 per cent. by volume of alcohol.

TYPE III

This type is represented by one strain received from California and one of reputed Spanish origin obtained from an Australian winery. They ferment maltose strongly, galactose more slowly and raffinose in part, in addition to glucose, fructose, mannose and sucrose. Neither of these strains has yet formed a vigorous or stable film on wine containing 14 per cent. of alcohol.

Of the strains studied, those belonging to the first group obviously conform very closely to *Saccharomyces beticus* Race α of Marcilla, and those of the second group resemble *S. beticus* Race β of the same author. The third group, which includes Cruess' strain No. 3 of his Group C. may, as implied by the Californian authors, represent strains of *Saccharomyces ellipsoideus* with the film-forming character more strongly developed than is usual in that species. This third type also conforms, at least in its fermentation characteristics, to the flor which Frolov-Bagreev claims to have obtained from Spain in 1908.

PLATE 7, 8 and 9. Successive stages in growth of flor on wine.

Plate 7. 12 days after inoculation. Plate 8. 19 days after inoculation.

Plate 9. 28 days after inoculation.

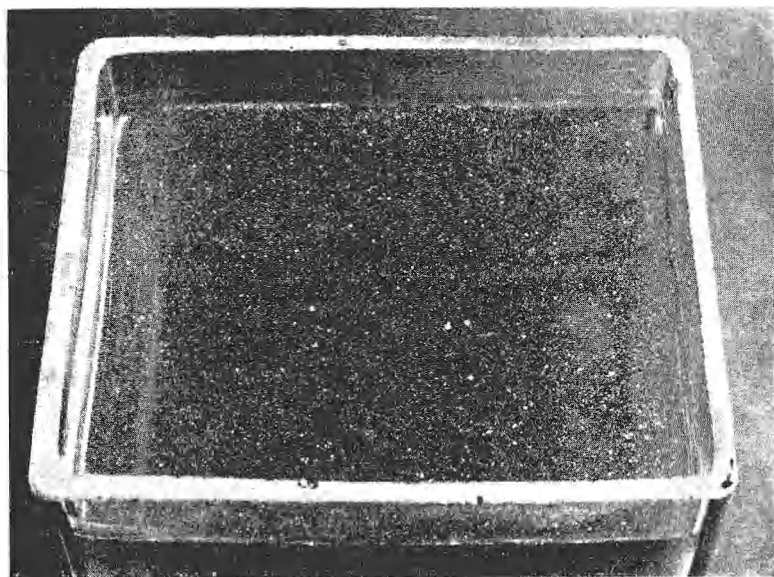


Plate 7. 12 days.

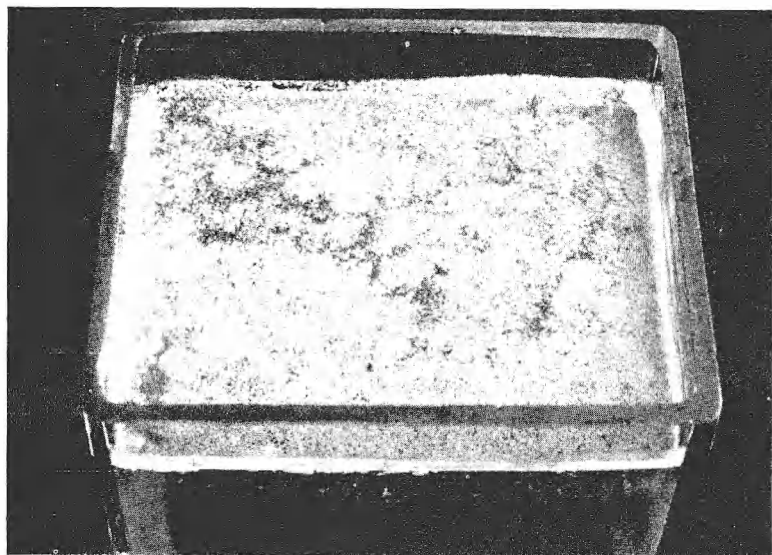


Plate 8. 19 days.

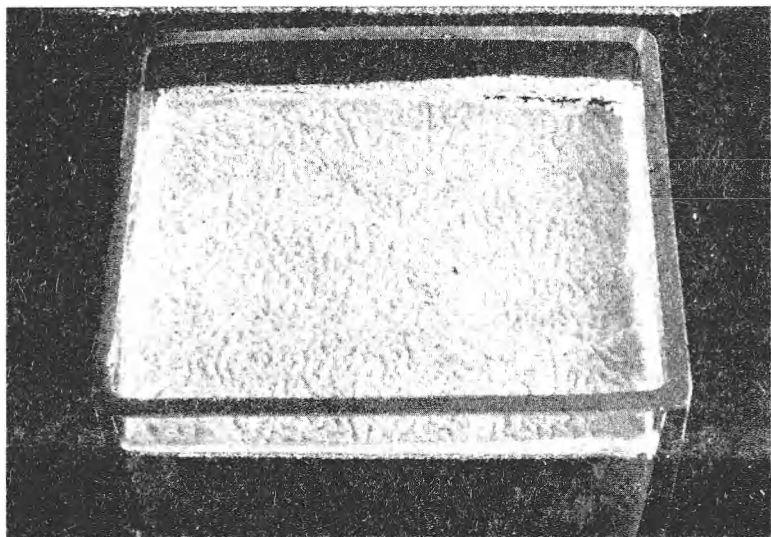


Plate 9. 28 days.

CHAPTER IV

FERMENTATION OF GRAPE JUICE BY FLOR YEASTS

It has already been mentioned that the flor yeasts bring about a vigorous alcoholic fermentation when seeded in grape juice. This fact has been noticed by many investigators. Schanderl (1936) found that the flor yeasts readily produce up to 15 per cent. by volume of alcohol when fermenting grape juice. Allan (1938) fermented grape juice with three strains of flor yeasts and three typical wine yeasts (*Saccharomyces ellipsoideus*) and found no significant differences between the two types of yeast as regards rate of fermentation, alcohol produced, volatile acid produced or sugar consumed.

Marcilla *et al.* (1939) found that the typical flor yeasts fermented grape juice vigorously and tolerated high concentrations of sugar well. He records fermentation of musts containing over 30 per cent. of sugar. His strains were not exceptionally high producers of alcohol, however, and did not produce more than 14 per cent. by volume of alcohol. Marcilla did not publish direct comparisons of fermentation by flor yeasts and other yeasts, but the results published for the flor yeast are quite normal fermentation figures. He mentions, however, that the flor yeast fermentations frequently give rather high values for volatile acidity.

Hohl and Cruess (1939) and Cruess (1948) found the rate of fermentation by flor yeasts to be very similar to that of fermentation by Champagne and Burgundy yeasts. The flor yeasts studied by these workers were also high producers of alcohol, some strains producing up to 18 per cent. by volume in direct or ordinary fermentations and 19 per cent. in syrupe fermentations.

The flor yeasts studied in our investigations have all fermented grape juice vigorously, but as with ordinary wine yeasts, the alcohol-forming ability varies somewhat from one

strain to another as does also the tolerance to high concentrations of sugar. In order to compare the fermentations brought about by a typical flor yeast and by ordinary wine yeasts (*Saccharomyces ellipsoideus*) a series of fermentations of grape juice were carried out in the laboratory.

Nine 13-oz. clear glass bottles, each containing 250 ml. of cleared Pedro juice and fitted with cotton plugs were sterilized in the steamer. Three bottles were then seeded each with 5 ml. of a 3-day culture of the sherry flor yeast (J7B) in similar grape juice and likewise three bottles were seeded with a Port yeast (No. 350) from our collection and three were seeded with a Champagne yeast (No. 352). All bottles were incubated at 20° C. All bottles were weighed immediately after inoculation and at intervals during the course of the experiment, and observations were made to detect any differences in the appearance of the fermentations or in the clarification of the liquid at the end of fermentation.

Hydraulic fermentation valves were fitted to the bottles after fifteen days when the vigorous fermentation had subsided and after 33 days of incubation when fermentation had ceased in all bottles the contents of the culture bottles were decanted and analysed.

Fermentation had commenced in all bottles within twenty-four hours and appeared to be quite normal and vigorous in all of them. The completion of fermentation, as indicated by absence of bubbles and by the loss in weight of less than 0.1 grm. in twenty-four hours, was reached rather earlier in the flor yeast than either of the others. The liquid in all bottles cleared well after fermentation, but the deposit of the flor yeast was more voluminous and more easily disturbed in decanting than were the deposits of the other yeasts.

The results of the analysis of the fermented liquids are shown in Table 1.

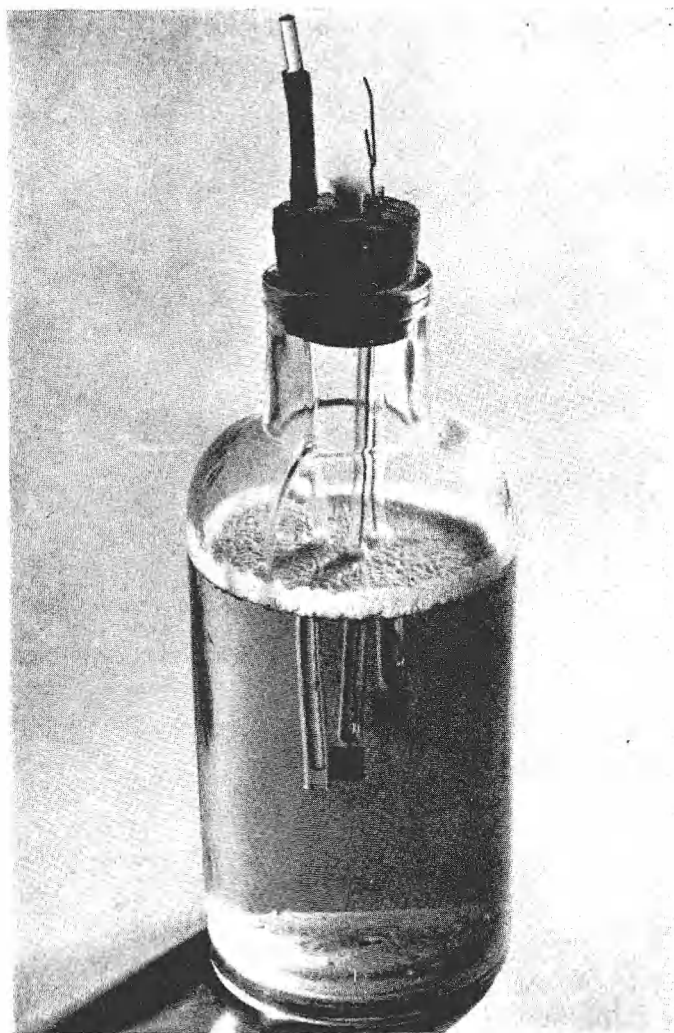


PLATE 10.

Culture bottle as used in laboratory experiments with flor yeasts.

TABLE I

Comparison of the fermentation of grape juice by a typical flor yeast, a Port yeast, and a Champagne yeast at 20° C.

Original juice. 24.6 per cent. reducing sugar, pH 3.52 and total titratable acidity 74.1 m.eq. or 5.55 gm./l. as tartaric acid.

Bottle No.	Flor yeast J7B			Port yeast 350			Champagne yeast 352		
	1	2	3	4	5	6	7	8	9
Days to complete fermentation	20	25	20	33	30	33	28	33	30
Alcohol, vol. % Pycnometer	14.7	14.7	14.7	14.7	14.5	14.4	14.5	14.5	14.5
Reducing sugar, gm./100 mls.	0.198	0.205	0.194	0.418	0.293	0.384	0.260	0.448	0.264
Sugar consumed, gm./100 mls.	24.4	24.4	24.4	24.2	24.3	24.2	24.3	24.2	24.3
Gm. sugar used to give 1% by vol. alcohol	1.66	1.66	1.66	1.65	1.68	1.68	1.68	1.67	1.68
Total titr. acidity, m.eqs.	80.5	80.5	80.5	78.0	78.5	77.5	75.5	75.5	75.5
Total titr. acidity, gm./l. tartaric	6.04	6.04	6.04	5.85	5.88	5.81	5.66	5.66	5.66
Vol. acidity, m.eq.	11.6	12.7	10.6	10.8	7.9	9.2	8.6	12.5	7.5
Vol. acidity, gm./l. acetic	0.70	0.76	0.64	0.65	0.47	0.55	0.52	0.75	0.45
Fixed acidity, m.eq.	68.9	67.8	69.9	67.2	70.6	68.3	66.9	63.0	68.0
Fixed acidity, gm./l. tartaric	5.17	5.08	5.24	5.04	5.29	5.12	5.02	4.72	5.10
pH	3.68	3.68	3.68	3.68	3.68	3.68	3.69	3.69	3.69
Glycerol, gm./l.	8.97	8.29	8.29	7.00	7.05	6.96	6.27	6.56	6.68
Aldehyde as p.p.m.	50	26	79	20	20	18	19	20	19

The only difference between the three yeasts as shown by these analyses, and which is statistically significant, is the slightly greater production of glycerol by the flor yeast. However, the amount of glycerol formed by ordinary wine yeasts varies considerably, and the amount produced by the flor yeast is well within the normal range.

We see then that these analyses confirm the results of other workers and indicate that the flor yeasts resemble typical wine yeasts, *Saccharomyces ellipsoideus*, in their fermentation of grape juice.

GROWTH AND METABOLISM OF THE
FILM STAGE

To the winemaker, the fermenting stage of the flor yeasts is of only secondary interest, for this part of the process of making sherry, that is, the conversion of grape juice to wine can also be brought about by other wine yeasts. It is the film stage of the flor yeasts which imparts the so-called 'flor character' to the wine, and which is accordingly of chief interest to the winemaker.

Anyone who has had practical experience in making flor sherry cannot fail to have noticed the wide variations which occur in the growth and metabolism of the flor film under winery conditions. Variations are common between films on different wines and even between those in different casks of the same wine or grown at different times on the same wine. Variations are noticed in the speed and vigour of film growth, in the appearance of the films and in the changes brought about in the composition of the wine.

1. APPEARANCE OF FILMS GROWING ON WINES

The films may be thin and smooth or somewhat granular in appearance or they may be thick and much wrinkled (see Plate 5). They may be moist or dry in appearance and the colour ranges from almost white through cream and grey to brown. They may be very unstable so that they break and sink in the wine very readily, or they may be relatively stable so that a cask can be lightly jolted or a culture flask carried about in the laboratory with little damage to the film. Such differences in the appearance of films are sometimes due to differences between strains of the flor yeasts, but to a far greater extent they are due to differences between the composition of the wines and the conditions under which the films are growing, as well as to differences between the ages of the films.

The appearance of the film in the early stages of growth depends on the way in which the wine has been inoculated. If, as is often the case, large portions of film have been transferred from another cask and carefully floated on the surface of the wine, these portions of film remain as opaque patches which give rise to new growth round their margins. The new growth is at first thin and lace-like, but gradually becomes thicker and opaque and extends till the whole of the surface of the wine is covered. On the other hand, if the wine is seeded with a suspension of flor yeast which has been well shaken so as to break up clumps of cells, then growth usually starts as a thin lace-like film which covers the whole surface of the wine before becoming thick enough to be opaque and easily seen. Under favourable conditions the film soon increases in thickness and becomes much wrinkled and cream to light grey in colour. Successive stages in film growth on wine are shown in Plates 7, 8 and 9. If conditions for growth are not favourable the film, of course, grows more slowly and it is also less wrinkled and in some cases may remain almost smooth.

As already mentioned, the colour of the flor growth on wine also varies from almost white to brown. Not infrequently winemakers who have noticed the presence of dark-coloured films on wines, sometimes intermingled with patches of lighter colour, have thought the different colours to represent different strains of flor. In reality, however, the colour of the film is usually a reflection of the conditions under which it has been grown. Flor films are commonly dark in colour when grown under unfavourable conditions and in extreme cases may be dark brown. High temperatures, insufficient oxygen in the headspace, excess tannin in the wine and high alcoholic strength are among the factors which have been found to lead to the growth of dark films most frequently. On the other hand very heavy, pale, almost white films which are unstable, are commonly associated with very rapid growth such as occurs on wine which has received an addition of too much ammonium salts or some other nitrogen source.

The presence of light and dark patches of film on wine is often due to changes in temperature. The film may become dark in colour and portions of it sink in the wine because of high temperatures during the summer months. Then in the autumn, when the weather is cooler, fresh growth starts and the gaps in the old film are filled with patches of young light-coloured film. Films which are stable usually have a dry appearance or sometimes have a waxy lustre, but films which appear to be moist are nearly always unstable and they readily break and sink in the wine. These latter include both light-coloured films which have grown on wines rich in nitrogen and also thin dark-coloured films which have grown under unfavourable conditions.

During the growth and life of the flor on wine, clumps of cells are continually becoming detached from the under-surface and sinking to the bottom of the cask or other container, where they gradually accumulate as a bulky flocculent deposit. For a period the rate of formation of new cells in the film is at least sufficient to keep pace with this deposition so that the film remains wrinkled and of more or less constant thickness. It may remain in this condition for a long period if growing on a large depth of wine such as in a puncheon, if the temperature and other conditions remain favourable, and more particularly, if portion of the wine is carefully drawn off and replaced with young wine of similar type as is done in the Solera system of Jerez de la Frontera. Eventually, however, unless the wine is replenished from time to time in this way, there comes a time when the wine has been so altered in composition that it is no longer a favourable medium for film growth, and so the production of new cells cannot keep pace with the deposition of old ones from the lower surface of the film. Consequently, the film becomes thinner, smoother and moister, until finally, large sections of it become submerged and sink to the bottom of the container, leaving the surface of the wine uncovered. Usually, the old thin films are dark in colour, but occasionally they are almost white, depending on the composition of the wine and other factors.

TABLE 2
The composition of wine before and after the growth of flor.
 Incubated at 20° C. for 90 days.

Original Wine	Strain J7		After growth of flor Strain 340		Strain 341	
Alcohol (Ebullioscope), % by v.	14.7	13.9	13.7	14.0	14.0	13.7
Alcohol (Ebullioscope), % P.S.	25.7	24.3	24.0	24.5	24.5	24.0
Glycerol, gm./l.	7.84	4.90	4.95	5.85	5.52	5.73
Titratable acidity, m.eq.	108	95	101	94	96	97
Titratable acidity, gm./l. tartaric	8.1	7.1	7.6	7.05	7.2	7.3
Volatile acidity, m.eq.	6.6	7.0	11.0	2.6	2.6	1.8
Volatile acidity, gm./l. acetic	.39	.42	.66	.16	.16	.11
Fixed acidity, m.eq.	101.4	88	90	91.4	93.4	95.2
Fixed acidity, gm./l. tartaric	7.6	6.6	6.75	6.85	7.0	7.15
pH	3.02	3.02	3.02	3.02	3.02	3.02
Total aldehyde, mgrm./l. acetaldehyde	61	418	414	275	283	362
Free aldehyde, mgrm./l. acetaldehyde	58	378	383	246	240	304

TABLE 2 (continued)

The composition of wine before and after the growth of flor.

Incubated at 20° C. for 90 days.

Original Wine	After growth of flor									
	Strain J7					Strain 340				
	222	263	263	211	211	211	211	202	232	341
Volatile esters, m.eq.	90	222	145	147	143	186	186	178	204	217
Volatile esters, mgrm./l. ethyl-acetate	140	222	145	147	143	186	186	178	204	217
Total reducing matter, grm./l.	90	222	145	147	143	186	186	178	204	217
glucose	140	222	145	147	143	186	186	178	204	217
Fermentable sugar, grm./l.	90	222	145	147	143	186	186	178	204	217
glucose	140	222	145	147	143	186	186	178	204	217
Lactic acid, m.eq.	0.65	0.10	0.08	0.08	0.13	0.15	0.15	0.26	0.31	0.15
Lactic acid, grm./l.	7.1	9.1	8.8	8.8	7.3	10.5	10.3	8.45	8.0	8.1
Tartaric acid, m.eq.	0.64	0.82	0.79	0.79	0.66	0.95	0.93	0.76	0.72	0.73
Tartaric acid, grm./l.	39.6	34.0	34.0	34.0	34.0	32.0	33.4	33.4	32.7	33.0
Tartaric acid, m.eq.	2.97	2.55	2.55	2.55	2.55	2.40	2.50	2.50	2.45	2.47
Malic acid, m.eq.	23.9	17.9	19.8	19.8	20.3	18.8	19.7	19.8	16.7	19.2
Malic acid, grm./l.	160	120	133	133	136	126	132	133	112	129
Acetylmethyl carbinol, qualitative test	—	—	—	—	—	—	—	—	—	—
Acetal	—	—	—	—	—	—	—	—	—	—
Dihydroxyacetone	—	—	—	—	—	—	—	—	—	—

Strong positive reaction

Trace

Positive test

2. THE CHANGES BROUGHT ABOUT IN WINES BY THE FLOR FILM

As pointed out in the preface to this publication, vigorous growth of flor film on wines does not always lead to the production of flor sherry. It has been found that wines carrying flor films in hogsheads sometimes develop appreciable flor character within three to six months, while sometimes this only happens after two to three years, and sometimes only slight development of flor character is noticed after even longer periods. Such differences in the response of wines to the flor process are, of course, of great importance to the winemaker, and in fact, they have constituted the chief problem in the production of flor sherry in this country. In order to gain information which would enable the winemaker to exercise better control over the process, it has been necessary to determine what changes the flor film brings about in the composition of the wine, which of these changes are most closely related to flor character as determined by competent winetasters, and the effects of various factors on such changes.

The changes brought about in a dry white wine by three different strains of flor yeast in laboratory cultures at 20° C. are shown by the analyses in Table 2. These cultures were grown in 32-ounce bottles each containing 800 ml. of wine. The depth of the wine was 12.6 cm. (4.95 inches) and the surface area was approximately 63.6 sq. cm. (9.7 square inches). The bottles were closed with waxed corks, each pierced by a short length of glass tubing 6 mm. (about $\frac{1}{4}$ inch) internal diameter, which was loosely plugged with cotton-wool to allow ventilation of the headspace (see Plate 10).

Further data concerning the effects of the flor film on wines, this time in casks under normal winery conditions, are provided by the analyses shown in Table 3.

From these results it can be seen that the wines have changed in composition under the influence of the flor. There has been a decrease in the specific gravity of the wines and also in the amounts of ethyl alcohol, glycerol, fermentable sugar, volatile acids and fixed acids which they

TABLE 3

Changes brought about in the composition of wine by the flor under winery conditions.

Six hogsheads of about 65 gallons capacity each containing about 53 gallons of wine.

	Original Wine	12 months after being seeded with flor (Means)
Specific gravity at 20° C.	0.9895	0.9880
Alcohol, per cent. by volume	14.5	14.1
Alcohol, per cent. proof spirit	25.4	24.7
Glycerol, grm./l.	7.9	4.6
Titratable acidity, m.eq.	82.5	77.0
Titratable acidity, as grm./l. tartaric	6.2	5.75
Volatile acidity, m.eq.	8.0	4.0
Volatile acidity, as grm./l. acetic	0.48	0.24
Fixed acidity, m.eq.	74.5	73.0
Fixed acidity, as grm./l. tar- taric	5.6	5.5
Tartaric acid, m.eq.	28.4	27.6
Tartaric acid, grm./l.	2.13	2.07
Malic Acid, m.eq.	14.6	14.0
Malic acid, grm./l.	0.98	0.94
Lactic acid, m.eq.	8.7	5.2
Lactic acid, grm./l.	0.78	0.47
pH	3.23	3.21
Total SO ₂ , mgrm./l.	62	19
Free SO ₂ , mgrm./l.	10	trace
Reducing matter, as grm./l. glucose	1.97	1.93
Fermentable sugar, as grm./l. glucose	0.79	0.10
Non-fermentable reducing mat- ter, as grm./l. glucose	1.18	1.83
Total aldehyde, as mgrm./l. acetaldehyde	37	444
Free aldehyde, as mgrm./l. acetaldehyde	37	379
Volatile esters, m.eq.	1.2	2.3
Volatile esters, as mgrm./l. ethylacetate	106	202
Neutral esters, m.eq.	2.48	3.3
Neutral esters, as mgrm./l. ethylacetate	218	290
Acetylmethylcarbinol, qualita- tative test	—	strong positive reaction

contain. On the other hand the analytical figures for certain constituents show increases because these have been produced by the flor. In this latter category are aldehydes, volatile esters, neutral esters, acetylmethylcarbinol, dihydroxyacetone, diethyl acetal.

The decrease in ethyl alcohol is always greater in small laboratory cultures than in casks, and has been found to be due in part to evaporation and in part to the activity of the flor. Under industrial conditions this decrease in alcohol may represent quite a serious loss, particularly if the wine is shallow, if ventilation of the headspace is excessive and if temperatures are high. On the other hand when the wine is in hogsheads or puncheons not less than about nine-tenths full and ventilation of the headspace is restricted, the loss of alcohol is never great and is often hardly detectable over periods of more than a year. In fact, when stored in dry cellars some casks of wine have actually been found to increase slightly in alcohol content because the wooden staves of the casks are more permeable to water than to alcohol, and so the wine loses water more rapidly than alcohol in a dry atmosphere where evaporation from the outside of the staves is rapid. On the other hand, if the casks are stored in a moist atmosphere, the loss of water is retarded and consequently the wine falls in strength.

A decrease in glycerol content of wines has been found to be a normal accompaniment of flor growth. Thus like alcohol, glycerol is formed by the flor yeast during the fermentation stage and is subsequently attacked by the film stage of the same organism. At present the products formed from glycerol are not fully known, but dihydroxyacetone appears to be one of them.

A fall in volatile acidity normally occurs in wines as a result of flor activity, but as will be explained later, laboratory cultures may, under certain conditions, undergo a subsequent rise in volatile acidity. The two cultures of strain J7 (Table 2), which had increased in volatile acidity at the end of the experiment, contained only 0.26 and 0.19 gm. per litre, respectively, of volatile acid when samples were examined six weeks earlier.

A fall in fixed acidity is also a normal feature of wines carrying flor films, and commonly amounts to between half and one gram per litre expressed as tartaric acid. It is partly accounted for by the decrease in tartaric and malic acids, but the analyses have not been sufficiently detailed to enable the changes in the amounts of the different acids in the wines to be presented in the form of a balance sheet. The pH of the cultures in Table 2 did not change during the experiment, but in other experiments changes in pH have been found to occur. Such changes are usually small and fall within the range $+0.1$ to -0.1 pH units, although greater changes have occasionally been observed. For example, wines to which ammonium salts have been added may decrease in pH value by 0.1 to 0.4 , depending on the amount of ammonium salts added and the buffer capacity of the wine.

The changes observed in the amounts of sugar present in wines during flor development depend very largely on the way in which the sugar is determined. If the total material capable of reducing Fehling's solution is determined in de-alcoholized wine, and is called reducing sugar, as is usually done, then little if any fall is noticed, and in some cases there is even an apparent increase in sugar. Thus Marcilla (1939) found that the flor film had little effect on the sugar content of several wines which he examined. However, if the actual sugar fermentable by yeast is measured by determining the reducing matter present before and after fermentation with a suspension of yeast under standardized conditions, then it is found that this fermentable sugar always decreases as a result of flor activity, and eventually disappears almost entirely. The discrepancies between the changes commonly observed in the fermentable sugar and in total 'reducing sugar', and which are shown in Table 2, are due to the production of acetylmethylcarbinol and possibly other reducing substances by the flor, which are not completely removed by evaporating to quarter volume as recommended in the A.O.A.C. official method for sugar determination in wines.

Of the substances formed by the flor when growing on

wines, the most characteristic, next to CO_2 , is acetaldehyde, but the amount formed varies very greatly according to the strain of flor used, the composition of the wine, and other factors. These will be discussed in greater detail later. The increase in aldehyde content is accompanied by increase in the amounts of several secondary products of acetaldehyde, notably acetylmethylcarbinol or acetoin, diethyl acetal and ill-defined compounds resulting from the combination of aldehyde with tannins and other polyphenols. The total volatile esters have been found to increase in amount during flor growth but the amounts formed have usually been less than reported by Marcilla. Another difference between the changes observed in Australian wine during flor growth and those reported by Marcilla for Spanish wines, is the greater production of lactic acid reported by Marcilla. Whereas no really significant increases in lactic acid have been observed in Australian wines carrying flor cultures of undoubted purity, very slight increases have been noticed in some casks in wineries. Marcilla, on the other hand, found the lactic acid content of wine to increase by as much as 0.5 gm. per litre in six months with what he believed to be a pure culture of flor. The results published by Bobadilla (1949) however, indicate that production of lactic acid by the flor yeasts is certainly not the general rule in Spain. In fact, he records that lactic acid may be consumed by the flor yeast with production of acetic acid, a finding which is in agreement with results obtained here when the flor has been grown on wines or artificial media with high lactic acid content. Saenko (1945) also reports that the strains of flor used in Armenia reduce the lactic acid content of wines which have undergone lactic acid spoilage, and recommends their use for this purpose. The available evidence, therefore, suggests that the increase in the lactic acid content of wines reported by Marcilla, and the relatively large amounts of lactic acid which have been found in some Spanish sherries, are not due to the activity of pure cultures of flor yeasts but rather to bacterial action on residual sugars and perhaps on malic acid.

In addition to the products which are found in the wines

and which are listed in Tables 2 and 3, numerous analyses of the gases in the headspace above the film in laboratory cultures and in casks in wineries have shown that carbon dioxide is a constant product of the film when growing on wines or on artificial media.

Another effect of the film on the wine concerns the oxidation-reduction potential or redox potential. This value, which is a measure of the oxidising or reducing intensity of the wine commonly reaches about 400 to 420 millivolts in wines left exposed to the air, as happens with wine seeded with sherry flor. Because of oxidation by the air, values of this order are reached in laboratory cultures and sometimes in the winery before there is much film growth. As the film develops, however, reducing conditions are set up in the wine and the redox potential gradually falls for a period and to a value which varies according to the composition of the wine and other conditions. The lowest values reached in wine in laboratory cultures are commonly between 220 and 280 millivolts, but values as low as 120 millivolts have been recorded. Only a few determinations of redox potentials of wine under flor in casks have been made. The readings indicated a fall in potential during flor growth but gave no indication of the lowest values reached. After the minimum potential has been maintained for a period, which varies from a few days to a few weeks in laboratory cultures, the conditions in the wine become less reducing and the potential rises again till eventually it reaches about 400 millivolts.

3. THE RELATIONSHIP BETWEEN FLOR CHARACTER AND ANALYSES

The ultimate criteria of flor character in wine are, of course, flavour, taste and aroma, but it is highly desirable to relate these qualities to chemical analyses in order to devise an objective measure for assessing experimental results and also to determine which changes are desirable and therefore to be encouraged, and which changes we should try to suppress. Anything approaching an exact chemical definition of flor character is hardly to be expected. The

chemistry of the flavours and aromas of wine is notoriously complex, for not only are many of the active substances very potent and thus effective in very high dilutions, but the effects of even major constituents such as alcohol, acid and sugar, depend not only on the absolute amounts of these which are present, but also on the proportion of one to another and on the presence of other substances which accentuate or mask certain flavours and aromas.

TABLE 4.

Order of flor character by tasting compared with aldehyde content of wines.

Wines in order of aldehyde content	Aldehyde mgr./l.	Order of preference by tasters				
		A	B	C	D	E
J7	506	J7	J7	J7	J7	J7
J3	425	J3	J3}	J3	J3	J19
J19	382	J19	J19}	J19	J19	J18
J5	266	J5} ¹	J5	J5}	J18	J3 ²
J18	256	J18}	J18	J18}	J5}	J5
J12	152	J12	J12	J12	J12}	J12

¹Brackets denote that samples were not considered to differ significantly as regards flor character.

²J3 was 'pointed down' by the taster because of 'coarseness'.

Search for correlation between flor character as detected by tasting, and changes in the composition of wines as indicated by chemical analysis, has been confined to the constituents enumerated in Table 2. During the course of the investigations a large number of wines have been submitted to panels of tasters who have been asked to place the wines in order of their flor character, disregarding as far as possible other characteristics of the wines. The results of such examinations have been compared with the analyses of the wines, and have shown a general agreement with the total aldehyde content of the wines. As would be expected, the correlation has been least regular when wines of different origin have been compared, but when different portions of the same wine have been seeded with different strains of flor yeast or have carried the flor film for different periods,

or when cultures on the same wine have been grown at different temperatures, then a close correlation has been noticed between the order in which the tasters have placed the wines and the order in which they are placed on the basis of aldehyde content. The results in Table 4 are typical of such comparisons and represent the placings by five tasters of six different portions of the same original wine which had carried films of six different flor yeasts for eleven weeks. The aldehyde contents of the wines when tasted are shown in the second column of the table.

Of course, the presence of acetaldehyde is not in itself sufficient to impart flor character to a wine. The simple addition of acetaldehyde to wine does not transform it into flor sherry, although it does give the wine some of the characteristics of flor sherry. If acetaldehyde and small amounts of diethyl acetal are added to the wine the result is nearer to flor sherry but it still not a very close approximation. The flor flavour and aroma are undoubtedly complex and depend on other things besides acetaldehyde and acetal. It is likely that acetylmethylcarbinol and, perhaps, other secondary products formed from the aldehyde as well as esters contribute to the characteristic flavour and aroma commonly referred to as flor character, but numerous tasting trials have shown that determination of the total aldehyde which has accumulated in the wine as a result of flor activity, provides a very useful indication of the development of flor character. In this connection it is interesting to note that the Russian workers (Saenko 1945; Frolov-Bagreev and Saenko 1945) have also used the accumulation of acetaldehyde as an indication of flor character, but have suggested that if this is used in conjunction with the ratio of acetal to aldehyde an even better indication is obtained. However, the figures which they publish for acetal content are far in excess of the theoretical equilibrium ratio of acetal to aldehyde, and, as the method of determining acetal is not stated, it seems likely that they have followed the early procedure of Trillat (1903, 1908) and included in this category the whole of the fixed aldehyde which is liberated by distilling in the presence of phosphoric acid. This fraction certainly

includes the acetal but it also includes aldehyde in combination with polyphenols as pointed out by Ribéreau-Gayon and Peynaud (1947). Although this fraction is probably of importance in sherry there is little evidence that it is directly under the influence of the flor yeast. The French workers have shown that a progressive increase in the fixed or combined aldehyde and a corresponding decrease in the free aldehyde occurs in sterilized wine stored in bottles closed with corks. In our investigations we have found that during the growth of the flor film on wine, fixed aldehyde increases as the total aldehyde increases, although not in a constant ratio, and that the conversion of free to fixed aldehyde sometimes continues after the wine has been separated from the flor film.

The importance of esters in wine is a subject on which opinions have changed within recent years. It was once widely believed that these substances played a major rôle in the flavour and aroma of wines, but modern work, particularly that of Genevois, Ribéreau-Gayon and Peynaud at Bordeaux (1947), has led to a modification of this view. It has been shown by Peynaud (1936) that the ester, ethyl acetate, is largely responsible for the characteristic flavour and aroma of wines spoiled by acetic acid bacteria, but it seems that the other esters in wine have only a minor effect on the quality of the product.

During the flor sherry investigations both the neutral esters extractable by petroleum ether, and the total volatile esters have been determined in wines before, during and after flor growth. Both of these fractions, particularly the neutral esters, have increased during flor growth. However, Heitz and his associates (1951) found an even greater increase in the amounts of neutral esters during the baking of California type sherries, so the amount of neutral esters obviously cannot be regarded as an indication of flor character, although it is likely that at least some of the esters included in this fraction contribute to the flavour of the wine. The amount of volatile ester is probably more important than the amount of neutral ester, but insufficient data have so far been accumulated to enable a conclusion to

be reached on this point. The amount of volatile ester is certainly less closely related to flor character than is the amount of aldehyde formed. Although the best wines in any series often contain over 250 p.p.m. of volatile ester expressed as ethyl acetate, wines with a high volatile ester content do not possess marked flor character unless they also have a high aldehyde content. A very high volatile ester content is usually associated with spoilage by acetic acid bacteria.

The actual amount of aldehyde necessary to impart flor character to wines varies considerably with different wines. Some have distinct flor character when they contain about 200 mgrm. per litre while in others this character is not appreciable until the total aldehyde reaches over 250 or even 300 mgrm. per litre. The aldehyde content of some wines reaches very high levels (over 500 mgrm. per litre) as a result of flor activity, so that the aldehyde dominates the flavour and aroma. Such wines are usually not pleasant to drink, but, if free from coarseness and other defects, they are very valuable for blending with wines of lower aldehyde content.

It appears from our results that aldehydes play a pre-dominating part in the flor flavour and aroma and that esters play a subsidiary rôle. Thus in the study of the factors affecting flor character development, special attention has been given to the effect of such factors on the accumulation of aldehyde in wines. This should not be interpreted as meaning that aldehyde accumulation is synonymous with flor character, however. After all, our objective is to produce sherry rather than aldehyde, and although experimental results have often been measured in terms of acetaldehyde, they have also been checked by tasting.

4. THE NATURE AND ORIGIN OF THE ALDEHYDE

By separating the aldehydes from flor sherry, oxidizing them to acids with dichromate and preparing the barium salts of these it was found that the amounts of barium in the salts corresponded with the composition of barium acetate. This would indicate that acetaldehyde is by far the most

plentiful aldehyde present, although it is likely that other aldehydes are present in small amounts and contribute to the flavour and aroma of the wine.

The most probable source of the acetaldehyde was considered to be oxidation of ethyl alcohol by the flor yeast cells and this hypothesis has been tested by growing the flor film on various artificial media of known composition and also by shaking various substrates with suspensions of flor yeast cells.

In artificial media without alcohol, film growth was never vigorous but slight film development occurred on such media with glycerol, acetate or lactate as carbon sources. Only traces of aldehyde (less than 5 mgrm. per litre) were produced on such media in the absence of alcohol, however, and when acetaldehyde was added to the media it disappeared in the course of a few weeks.

In an experiment using the artificial medium No. 9 (see Appendix) but without glycerol, slow film growth was obtained in the presence of 2, 1, 0.5, 0.2 and 0.1 per cent. of alcohol by volume. At the end of three weeks complete thin films were present on all cultures and the amounts of aldehyde present were as shown in Table 5:

TABLE 5.

	Culture No.				
	1	2	3	4	5
Original alcohol content per cent. by vol.	2	1	0.5	0.2	0.1
Aldehyde after 21 days mgrm./l.	41	31	10	nil	nil
Iodoform test after 21 days	+	+	+	—	—

At this stage all cultures were thoroughly shaken up so as to distribute the yeast cells throughout the liquid and cultures 4 and 5 were subdivided. To one portion of each of these (4a and 5a) acetaldehyde was added at the rate of 400 mgrm. per litre. The film growth and aldehyde content of these cultures after further periods of incubation were as shown in Table 6:

TABLE 6.

	Culture No.						
	1	2	3	4a	4b	5a	5b
After 6 days film growth as per cent. cover	80	60	25	10	trace	25	trace
After 6 days total aldehyde as mgrm./l.	52	28	15	31	nil	22	nil
After 12 days film growth as per cent. cover	100	100	90	50	trace	70	trace
After 12 days total aldehyde as mgrm./l.	47	25	nil	nil	nil	nil	nil

(uninoculated controls to which 400 mgrm./l. of aldehyde had been added lost 47 mgrm./l. in six days).

From these results it appears that the aldehyde arises from the alcohol and is itself utilized by the flor film. In other words it represents an intermediate product in the oxidation of ethyl alcohol by the flor film but under some conditions this intermediate accumulates in the medium.

Further data concerning the origin of the acetaldehyde have been obtained from experiments with suspensions of flor yeast cells. The cells have been grown in 100 ml. quantities of dilute grape juice medium in 20-oz. flat bottles incubated on their sides for 48 hours at 25° C. The cells were separated by means of a centrifuge, washed three times in sterile tap water and resuspended as a 10 per cent. w/v. suspension in M/20 phosphate buffer of pH 3.25. The suspensions were stored in the refrigerator till used, but were never kept longer than three days.

Experiments were carried out by adding 2 ml. of this suspension (200 mgrm. of yeast) to 8 ml. of different solutions in 8-oz. flat bottles fitted with screw caps or with rubber bungs pierced by glass capillary tubes with taps. The bottles were then placed flat on an electric shaking machine and shaken at 120 oscillations per minute for the duration of the experiment, after which the yeast cells were poisoned with silver sulphate as recommended by Janke and Kropacsy (1935) and were then centrifuged out and the supernatant liquid examined. The substrates treated in this way were ethyl alcohol, glucose, glycerol, lactate acid, acetic acid, tar-

taric acid, malic acid, citric acid, succinic acid and fumaric acid, but aldehyde was formed in appreciable amounts only in the presence of ethyl alcohol.

It seems then that the main source of the increase in aldehyde content of wines under the flor film is the oxidation of ethyl alcohol with the accumulation of acetaldehyde as an intermediate product. It has been found that because aldehyde is only an intermediate and not an end product, it never represents more than about 15 per cent. of the alcohol which has been oxidized by the flor, even during the periods of greatest aldehyde accumulation. The other 85 per cent. has been completely oxidized to carbon dioxide and water.

5. THE COURSE OF ALDEHYDE ACCUMULATION AND OTHER CHANGES

If flor film is grown on a wine for a long period and the aldehyde content of the wine is determined at frequent intervals, it is found that the aldehyde does not increase continuously during the whole of this period but fluctuates to a greater or less extent, depending on the wine, the strain of flor and the conditions of culture.

When studied in laboratory cultures of the type indicated on page 32, wines behave in different ways as regards aldehyde accumulation. In some wines the aldehyde increases steadily for about six to ten weeks reaching between 300 and 500 mgrm. per litre, remains at about this value with only slight fluctuations for a further six to eight weeks and then gradually declines. In other wines the aldehyde increases to between about 150 and 250 mgrm. per litre by the third to the fifth week, then falls to a low level, sometimes below the original value, by about the sixth week and, after a further period of a few weeks, rises again to a value of between 300 and 500 mgrm. per litre, which is usually sustained for several weeks before the level gradually falls again. Then again, there are wines in which the aldehyde content shows an initial rise followed by a fall, and thereafter remains low for a long period before any second rise becomes

apparent. Samples which have been withdrawn at intervals and tasted indicate that the flor character of the wine fluctuates in a manner which closely parallels the behaviour of the aldehyde. In Fig. 1 are shown curves representing the course of aldehyde accumulation in three different wines in laboratory cultures. These curves have been selected from a large number because they show three different types of aldehyde accumulation, but it should not be inferred that wines fall into three distinct classes in this respect. Other minor variations and intermediate types also occur.

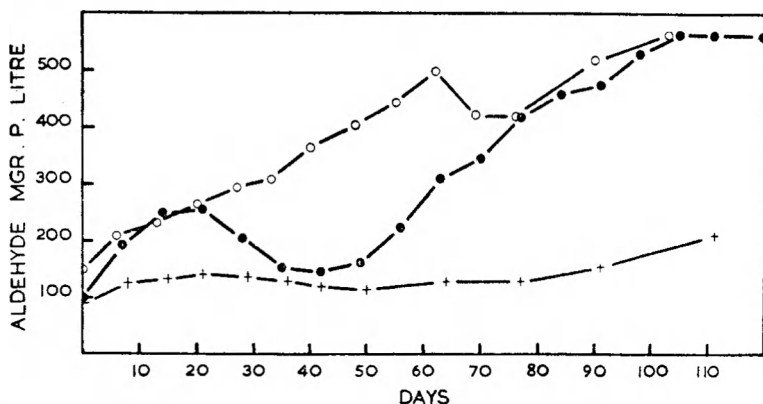


Fig. 1. Course of aldehyde accumulation in three different wines under sherry flor in laboratory cultures.

Actually the levels of aldehyde reached and the times occupied by different phases of the curve depend on the surface area and the depth of the culture.

Fig. 2 represents the accumulation of aldehyde in a wine in laboratory cultures of different depth but with the same surface area. It will be noticed that in this wine the aldehyde content of both cultures increased at first and reached a first maximum at about the fourth week. The time at which this maximum was reached was the same for both cultures and corresponded with the time of complete cover with thick, heavily wrinkled flor film. The amount of aldehyde present at this stage, however, varied according

to the depth of the culture and was greater in the shallow culture. In both cultures the aldehyde level decreased after the fourth week and subsequently rose again, and it is evident that the rate of this fall and of the second rise in aldehyde were greater in the shallow culture.

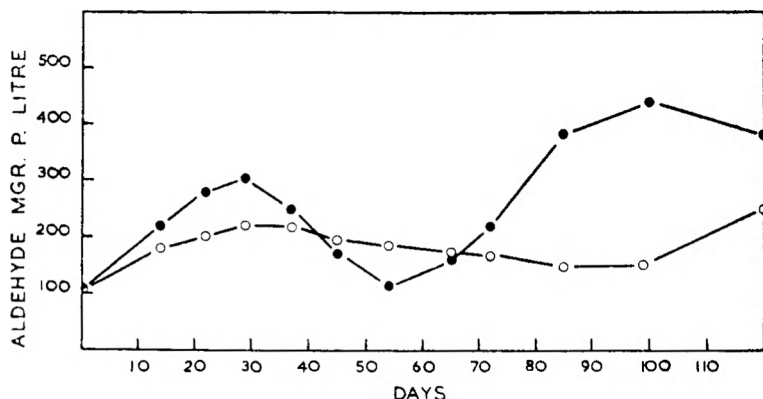


Fig. 2. Course of aldehyde accumulation in cultures of different depths. O ———— O 10 inch culture. ● ———— ● 2 inch culture.

Fig. 3 shows aldehyde accumulation curves for three different wines carrying flor films in hogsheads under winery conditions. In each case the cask contained about 50 gallons of wine, had 12 to 15 gallons ullage space, and was closed with a cotton bung. Here we have curves of essentially similar form to those shown in Fig. 1 representing laboratory cultures. With the smaller surface : volume ratio in the casks, however, the accumulation of aldehyde is much slower than in the shallow laboratory cultures and we see more clearly the importance of the differences in behaviour of different wines. In the small laboratory cultures those wines which increase steadily in aldehyde content reach a high level of aldehyde a few weeks ahead of the wines which fluctuate widely, but in casks the difference may amount to many months and is, therefore, of considerable industrial importance.

Besides aldehyde accumulation, other changes brought about in the composition of wines by the flor have been followed by analysis of samples drawn periodically during the life of the film. In this way the course of the changes brought about in aldehyde content, volatile acidity, fixed acidity, pH, redox potential, alcohol content, fermentable sugar content and volatile esters as well as oxygen consumption, have been determined in various experiments though never all in any one experiment. The course of some of these changes occurring in wine in laboratory cultures are shown in Figs. 4, 5 and 6. The other determinations listed in Tables 2 and 3 were made only at the beginning and the end of experiments.

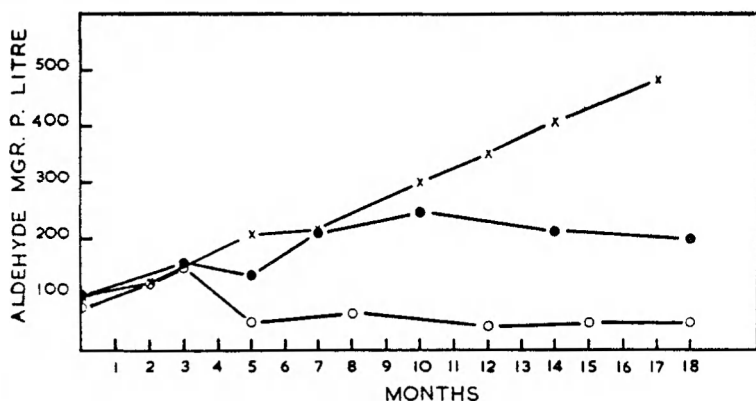


Fig. 3. Course of aldehyde accumulation in three different wines under sherry flor in casks.

Fig. 4 shows the changes which occurred in the amounts of aldehyde, volatile acid and fermentable sugar and in the redox potential over a period of 120 days in a laboratory culture of wine. This was one of a series of cultures each grown in 800 ml. of wine in a 32-oz. jar which was closed with a waxed cork pierced by a sampling tube, and a ventilation tube. The cork also carried two platinum electrodes dipping 6.5 cm. below the surface of the wine for the determination of redox potential. A KCl-agar bridge was inserted when such measurements were made. The level of the wine in the

cultures was maintained at a constant level throughout the experiment by adding wine from other similar cultures to replace the portions withdrawn periodically for analysis. Analyses were carried out on eight such cultures and although the actual values obtained on any one date differed somewhat for different cultures, the curves representing the changes in the wine were all of similar shape to those shown in Fig. 4.

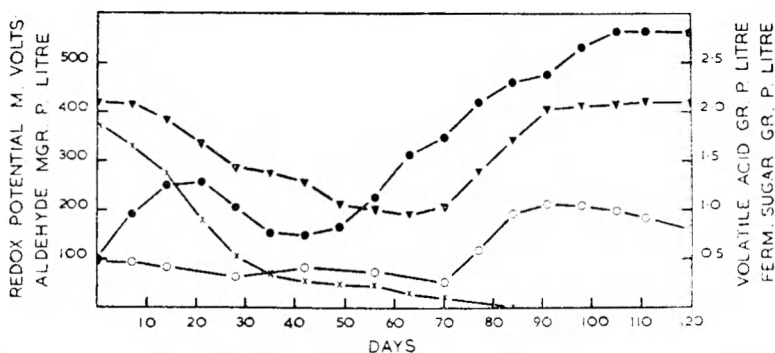


Fig. 4. Changes occurring in total aldehyde, volatile acidity, fermentable sugar and redox potential in wine under sherry flor in laboratory culture.

- ————— ● total aldehyde, mgrm. per litre.
- ————— ○ volatile acid, grm. per litre.
- X ————— X fermentable sugar, grm. per litre.
- ▼ ————— ▼ redox potential, millivolts.

Besides the fluctuations in aldehyde already mentioned, the other changes which occurred in the wine also differed at different stages in the life of the cultures. During the early period, up till the time of maximum film development, the aldehyde content increased from 107 to 257 mgrm. per litre. During the same period the redox potential fell from 420 to 338 millivolts, the volatile acidity fell from 0.49 to 0.37 grm. per litre and the fermentable sugar fell from 1.86 to 0.90 grm. per litre. From the third to the sixth week the aldehyde content fell to 148 mgrm. per litre, while the fermentable sugar and the redox potential continued to fall and the volatile acidity remained low. After the seventh

week the aldehyde content increased rapidly and this was followed about three weeks later by a marked rise in both redox potential and volatile acidity at a time when the fermentable sugar had almost entirely disappeared from the cultures.

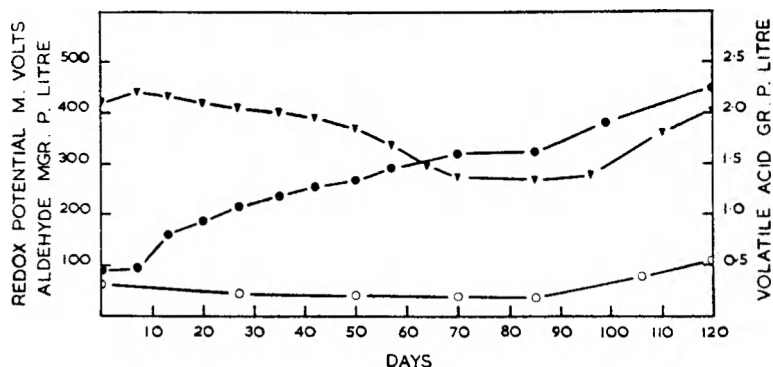


Fig. 5. Changes occurring in total aldehyde, volatile acidity and redox potential in wine under sherry flor in laboratory culture.

- — total aldehyde, mgm. per litre.
- — volatile acid, gm. per litre.
- ▼ — redox potential, millivolts.

The differences encountered in the course of aldehyde accumulation in different wines have already been mentioned. Differences have also been noticed in the curves representing changes in volatile acidity and redox potential in different wines although here the differences are in the values reached rather than in the shape of the curves. In Fig. 5 are shown the changes which occurred in the aldehyde content, volatile acidity and redox potential of a wine in which aldehyde accumulation followed a different course from that shown in Fig. 4. Here again, as in the first wine, we find that both the redox potential and the volatile acidity decreased during the early stages and subsequently increased again, but in the second wine the redox potential did not reach such low values as in the first.

In other experiments the oxygen consumption of the film has been followed by continuous passage of air through

the headspace above the film at a controlled rate and periodical analysis of the headspace gas. Knowing the rate of flow and the composition of the gas entering and leaving the culture, the oxygen consumption can be calculated. The results of such an experiment are represented by the curves in Fig. 6 which show the oxygen consumption in c.c. per day, as well as the changes in the aldehyde content and volatile acidity of a culture having a surface area of about 9.7 sq. inches (63.6 sq. cms.) and incubated for 98 days at 20° C. As can be seen from the graph, the rate of oxygen

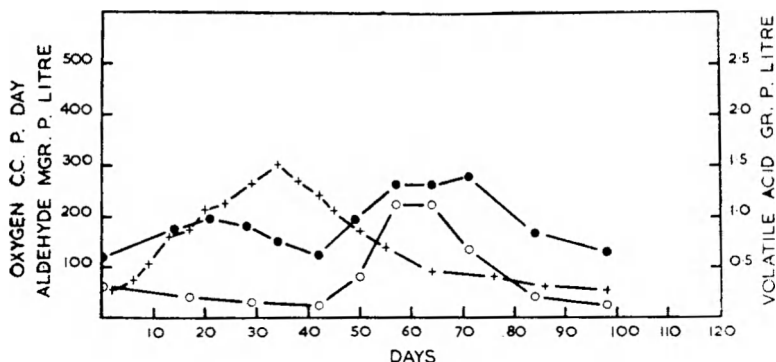


Fig. 6. Changes occurring in total aldehyde content and volatile acidity of the wine, and in the oxygen consumption of the flor in a laboratory culture.

- ——— ● total aldehyde, mgrm. per litre.
- ——— ○ volatile acid, grm. per litre.
- + ——— + oxygen consumption, c.c. per day.

consumption gradually increased, reaching a maximum during the fifth week and then declined rather rapidly till the ninth week and more slowly thereafter. The aldehyde content of the wine, after rising at first, was depressed during the period of maximum oxygen consumption but increased markedly as the oxygen consumption declined. The volatile acidity of the wine, which had decreased slowly during the early period, also increased appreciably for a few weeks when the oxygen consumption of the film declined.

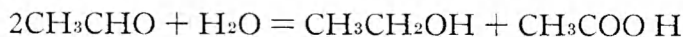
6. THE NATURE AND SOURCE OF THE VOLATILE ACID FORMED IN SOME CULTURES

Growth of the flor film on wines in laboratory cultures or under industrial conditions in wineries, is usually accompanied by a reduction in the volatile acidity of the wine. This phenomenon has been commented on by most of the people who have studied sherry flor experimentally (see Cruess and Podgorny, 1937; Schanderl, 1938; Bobadilla and Navarro, 1949; Marcilla, 1939; Saenko, 1945). Occasionally, however, it is found that the volatile acidity of a wine increases while it is under the flor film and it has been customary to ascribe such happenings to infection of the wine with vinegar bacteria (*Acetobacter*). While an infection of this type is certainly the cause of the increases in volatile acidity noticed in some wines, it does not explain all those which have been observed. Experiments with pure cultures in the laboratory have shown quite clearly that under certain conditions the flor yeast itself is able to bring about an increase in the volatile acidity of wine. Amounts of up to 2 grm. per litre (as acetic acid) have been formed in some cultures in the absence of any detectable infection with bacteria. By preparing the barium salt of the volatile acid and then determining the percentage of barium therein, it has been shown that the acid is chiefly acetic acid. Actually, Trillat and Sauton (1910) showed that yeasts are able to form acetic acid and ethyl acetate in the presence of alcohol and aldehyde and they believed that the acetic acid arose from oxidation of the aldehyde.

In our investigations we have found two sets of circumstances, either of which can lead to the production of acetic acid by the flor film when growing on wines.

(a) Anaerobic conditions. If the culture is placed under more or less anaerobic conditions by cutting off or at least greatly restricting the air supply after some considerable accumulation of aldehyde has already occurred, then the aldehyde disappears from the wine and the volatile acidity increases by an amount equivalent to about half the loss in aldehyde. It appears that under these conditions the flor

is able to bring about what is known as a dismutation reaction whereby a molecule of aldehyde is reduced to alcohol, and simultaneously another molecule of aldehyde is oxidized to acetic acid.



This type of dismutation is one of the well-known reactions of yeasts.

(b) Heavy film growth on shallow cultures. In laboratory cultures the early fall in volatile acidity of wine is commonly followed at a later stage by a rise. This rise is most pronounced in cultures carrying a heavy film growth and in shallow cultures. That is to say, it is most marked when the amount of yeast cell material per unit volume of wine is high and it seems likely that this volatile acid arises at least in part from the breakdown of yeast cell material. Support for this view is seen in the fact that addition of yeast extract to wines increases the amount of volatile acid formed by the flor when growing on them. Another possible source of volatile acid in these cultures is the destruction of lactic acid by the flor. In artificial media it has been found that the flor film destroys lactic acid with simultaneous production of acetic acid.

Although the production of volatile acid in wine by the flor has been clearly demonstrated in laboratory cultures not infected with *Acetobacter*, this is less easy to demonstrate under winery conditions. Up till the present any increases observed in the volatile acidity of wine in casks in which no bacteria could be found, have been very small. The ability of the flor to produce volatile acid although demonstrable in laboratory cultures, appears unimportant under industrial conditions, and when substantial increases in the volatile acidity of wine under flor are noticed in the winery, this should be taken as strong presumptive evidence of infection by *Acetobacter*.

Determinations of pH, fixed acidity and volatile esters of wines have not disclosed fluctuations comparable with those noticed in aldehyde, volatile acidity or redox potential. In general, the changes which occurred in pH, fixed acidity and volatile esters have increased with the duration of

experiments, although volatile ester production is often increased when volatile acidity increases and usually declines after very prolonged incubation of cultures.

The relationship of the various changes to one another and, in particular, to changes in aldehyde content, will be discussed more fully in a later chapter, but first, the effects of various factors on the growth and metabolism of the flor film will be discussed.

FACTORS AFFECTING THE GROWTH AND
METABOLISM OF THE FLOR FILM

THE differences which have been noticed in the appearance of the flor film and in its effects on wines, have already been mentioned. In this chapter we will consider the influence of various factors on the growth of the film and its metabolism in order to obtain some understanding of the causes of such differences. In view of the importance of acetaldehyde in flor sherry, it is particularly desirable to know something of the factors which influence aldehyde accumulation. In this connection the course of aldehyde accumulation, that is, the shape of the 'aldehyde curve', is considered at least as important as the actual quantity of aldehyde found.

The effects of various factors on the growth and metabolism of the film have been studied both in wine and in artificial culture media. Many experiments have been carried out using wine as a culture medium and modifying its composition in various ways, since these experimental conditions are more closely related to practical conditions than when artificial media are used. Sometimes, however, the experimental conditions can be more closely controlled and repeated if synthetic solutions of known composition are used instead of wine as culture media. Accordingly, many experiments have been carried out with synthetic media. However, since even synthetic culture media must be complex if they are sufficiently complete to support film growth, it has been found convenient to investigate and to confirm certain points by shaking simple solutions with suspensions of flor yeast cells as mentioned on page 43. Because the cells are not actually multiplying during such experiments, neither the experimental solution nor other conditions need be suitable for growth. This means that one can carry out experiments with one or more components of wine at a time.

Although, in practice, the various factors which influence the growth of the flor and its effects on wine are all more

or less inter-related, it is convenient to discuss them separately while at the same time pointing out such inter-relationships. For this purpose these factors will be grouped under the following headings:

1. The strain of flor.
2. The amount and nature of the inoculum with which the wine is seeded.
3. The environmental factors.
4. The composition of the wine.

1. THE STRAIN OF FLOR

The rate of growth, appearance and stability of the film and also the changes it brings about in wine, are all to some extent dependent on the strain of flor yeast used. However, the differences noticed between the films produced by different strains are in some instances due to differences in their tolerances for particular conditions. For example, two strains produced very similar growth on a wine containing 14 per cent. of alcohol (24.5 per cent. P.S.), while on a wine containing 15 per cent. alcohol (26.2 per cent. P.S.) one grew vigorously and produced a heavily wrinkled film, while the other grew more slowly and produced a thin, almost smooth film because it had a lower tolerance for alcohol than the former strain. Thus some of the most important differences between strains as regards rate of growth and stability of the film seem to be related to their tolerances and responses to various conditions. These will be discussed further when the other factors are dealt with.

Of the different strains of flor which have been studied, all have brought about essentially the same kind of changes when grown on wines, but details of these changes, such as the course of aldehyde accumulation and the rate at which different substances are attacked or produced, vary somewhat with different strains. Here again, as was pointed out previously in regard to film growth, it is likely that differences noticed are due at least in part to the variations in the tolerances of strains to particular conditions, but it does seem that some strains normally accumulate aldehyde more rapidly than do others. The results presented in Table 2 show the

general similarity in the changes which three strains of flor yeast brought about in a wine in the laboratory. As far as the accumulation of acetaldehyde is concerned, however, it is more instructive to compare curves representing the course of aldehyde accumulation throughout the period of the experiment. The curves shown in Fig. 7 represent the aldehyde accumulation in one of the cultures of each strain.

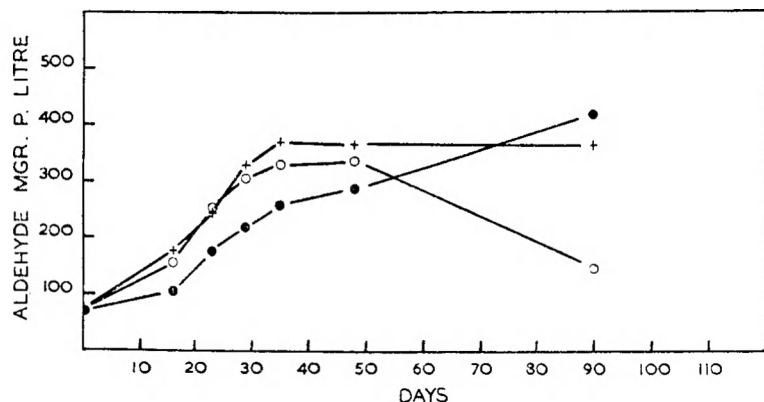


Fig. 7. Course of aldehyde accumulation in a wine under three different strains of flor in laboratory cultures.

● — strain J7; ○ — strain 340; + — strain 341.

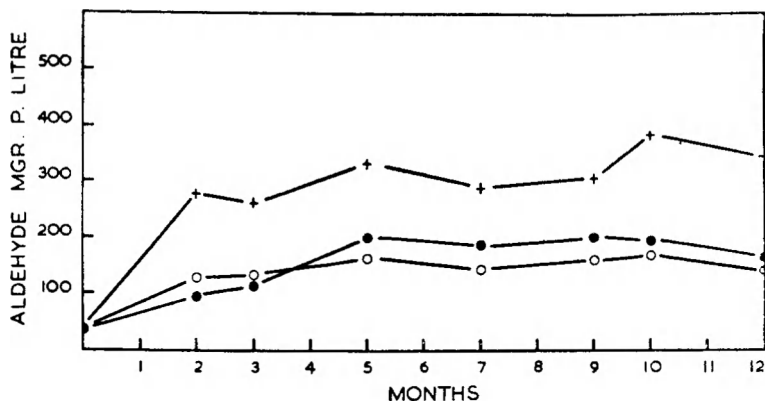


Fig. 8. Course of aldehyde accumulation in a wine under three different strains of flor in casks.

● — strain J7; ○ — strain 340; + — strain 341.

Fig. 8 represents the accumulation of aldehyde by these same three strains, but this time grown in hogsheads of wine in a winery. As can be seen from the figures, strain 341 accumulated aldehyde more rapidly than the other strains in both experiments. Actually these strains have been compared in two different wines under normal winery conditions, and in each case six hogsheads of wine were seeded with each strain, thus ensuring adequate replication to permit statistical analysis of results. As might be expected, results have varied somewhat from one cask to another, but in both wineries, as in the laboratory, strain 341 has accumulated aldehyde significantly faster than either of the other two strains.

2. THE AMOUNT AND NATURE OF THE INOCULUM

As might be supposed, the films cover the wine more rapidly when the amount of inoculum is increased, but the type of inoculum used and the method of inoculation also influence the time taken for complete cover. Most rapid and regular growth has been obtained when young vigorous film from a similar wine has been used as inoculum and portions of it floated on the surface of the wine to be seeded. Films which were several months old, have also been used successfully for seeding fresh cultures when still healthy in appearance, but generally, when old films have been used, there has been a longer lag period before vigorous new growth occurred. When wines have been seeded from very old dark-coloured films, growth has been very slow in starting.

Other types of cultures or stages of growth besides films may be used to inoculate wines. In the laboratory, the rates of film growth were compared in bottles of wine which had been inoculated with flor from the following different types of cultures, all grown at 20° C.

- (a) Streak culture on grape juice agar, 3 days old.
- (b) Fermenting culture in grape juice, 5 days old.
- (c) Grape juice which had undergone fermentation and on which a film had started to form.
- (d) Film culture on wine, 17 days old.

Two strains of flor yeasts were used and the experiment was replicated four times, with each strain giving eight cultures of each type, or thirty-two in all. From each of the thirty-two cultures, suspensions of yeast cells in sterile saline were prepared so that each suspension contained the same number of cells (50 million per ml.). 50 ml. portions of a sterilized dry white wine containing 14.6 per cent. of alcohol (25.5 per cent. P.S.) were placed in each of 32 four-ounce bottles and 1 ml. of the appropriate cell suspension added to each bottle. The bottles were closed with waxed corks through which a 3 mm. ($\frac{1}{8}$ inch) hole had been drilled and plugged with cotton, and were then incubated at 20° C. and the rates of film development observed.

Although the fermenting stage was the least satisfactory source of inoculum, film development was vigorous in all bottles and the differences noticed between the rates of growth from different inocula were small. It is also worthy of note that the addition of a suspension of flor yeast cells was quite a satisfactory method of inoculating the wine.

The effect on film-forming vigour of repeated culture of a flor yeast under conditions which do not allow film formation was investigated with the same two strains (J7 and J12) as were used in the preceding experiment. These two yeasts were subcultured repeatedly over a period of four months under the following conditions:

(a) Streak cultures on grape juice agar, subcultured every seven days and incubated at 20° C.

(b) Film culture on wine, subcultured every fourteen days and incubated at 20° C.

(c) Fermenting culture in grape juice, subcultured every seven days and incubated at 20° C.

(d) Fermenting culture in grape juice, subcultured every seven days and incubated at 30° C.

The cultures in grape juice (c and d) were always subcultured before fermentation had ceased and thus had no opportunity to form films during this period.

The experiment was replicated four times with each of the two yeasts so that four cultures of each yeast were subjected to each of the serial cultural treatments mentioned

TABLE 7

Film formation by two strains of flor yeasts after repeated cultivation under different conditions.

Previous cultural treatment	Strain J7		Time for complete cover in days				Mean
			Strain J7		Strain J12		
Streak culture on agar 20° C.	35	28	28	28	14	18	18
Film on wine 20° C.	18	21	21	28	14	18	15
Fermentation 20° C.	24	21	21	28	18	18	17
Fermentation 30° C.	28	28	28	28	21	28	30

(S.E. of difference between two means of four cultures = 2.7)

above. After these treatments had been continued for four months different portions of wine were inoculated with cell-suspensions prepared from the final cultures as in the previous experiment. The bottles of wine were incubated at 20° C. and the rates of film development observed. The results of this experiment are summarized in Table 7.

It appears from these results that the rate of film formation is affected by the previous cultural treatment of the flor yeast, particularly by repeated cultivation as a fermenting culture at a high temperature. A statistical analysis of the results in Table 7 shows that although the effect of cultural treatment was barely significant in the case of strain J7, it was highly significant, or, in other words, a very real effect in the case of strain J12.

The film-forming vigour of the various cultures mentioned above, was very easily restored, for when the different cell suspensions were added to bottles of sterilized grape juice, no differences could be detected between the rates of fermentation or subsequent film-formation by the different types of cultures.

It should be noted that the cultures used for inoculating the wines in the above experiment were all young and vigorous. It has been found that old cultures, particularly old cultures on agar media, are not suitable for inoculating wines directly, but are best seeded in flasks or bottles of grape juice closed with cotton plugs and allowed to carry out a fermentation at about 20° C. After fermentation is complete the flasks are left undisturbed for several weeks until a film develops on the surface of the fermented liquid. This film may then be used for inoculation of wine.

Since the common practice in Spain, as described by numerous writers, involves continuous use of the flor film on many successive batches of wine, it was considered desirable to compare the activity of flor film after being used many times, with that of young film. In laboratory cultures it was found possible to draw wine off from below the film and then refloat the film by the careful addition of fresh wine so that the film suffered very little damage. Three cultures of each of two strains of flor yeasts were treated in this way, so that each of the six films were used to treat

six successive batches of wines. Each batch of wine carried the film for a period of six weeks, and during the whole of the experiment very little film-drop and no appreciable darkening of the films occurred. The changes in aldehyde, volatile acidity and alcohol content of each batch of wine were followed during the treatment and it was found that under these conditions there was only slight loss in the activity of the films during the course of the experiment.

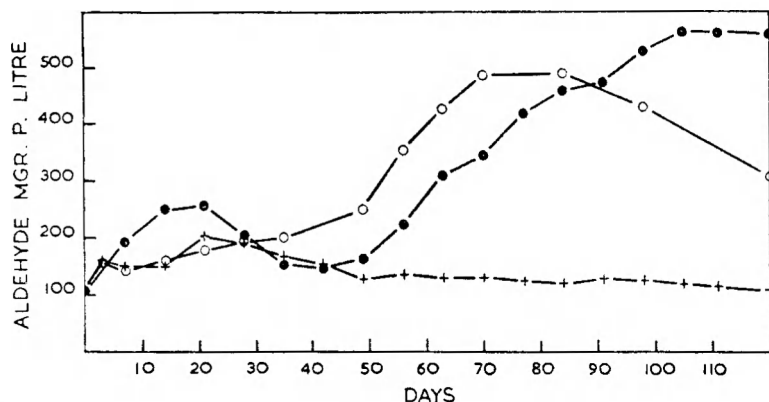


Fig. 9. Course of aldehyde accumulation in a wine when placed under flor films of different age.

- — ● wine freshly seeded with flor.
- — ○ wine placed under a complete flor film which had grown for 4 weeks on a previous culture.
- + — + wine placed under a complete flor film which had grown for 7 months on a previous culture.

However, in experiments where films have been allowed to remain on one batch of wine for a long period, until the film had become dark in colour, and then refloats on a fresh batch of wine, a considerable loss of activity has been noticed. In Fig. 9 are shown curves representing the aldehyde accumulation in different jars of the same wine, one of which was seeded with flor in the ordinary way; in the second the wine was placed under a complete but young film (four weeks old); and in the third, the wine was placed under a film which had previously remained for seven months on one 800 ml. batch of similar wine and had become

dark-grey in colour. Actually, each treatment was applied to three jars of wine and the results of the triplicate jars were very similar in each treatment. For clarity only one culture of each type is represented in the figure.

As can be seen from the curves, aldehyde accumulation in the culture seeded with flor in the ordinary way, rose to a first maximum at two to three weeks, then fell and subsequently rose again. The cultures which started with young complete films did not show the early peak in aldehyde, but after a slow initial rise increased rapidly in aldehyde, and from the fifth to the twelfth week contained appreciably more aldehyde than the first group of cultures. When we come to the cultures which commenced the experiment, as seven-month-old films, however, we notice a very marked fall in activity. The aldehyde content of these cultures after a small increase during the first few weeks, subsequently decreased and remained at a low level for many weeks. Although the graph only shows the course of aldehyde accumulation for 120 days, these particular cultures were kept for a further five weeks, and by that time two of them had commenced to increase in aldehyde content.

3. THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE GROWTH OF FLOR FILM

(1) *Oxygen supply*

One of the most important factors influencing film growth on wines or other media is the air supply, or more exactly, the proportion of oxygen or the oxygen tension in the atmosphere immediately above the surface of the wine. As previously mentioned, it was pointed out by Hansen over 60 years ago that oxygen is one of the basic requirements of yeasts growing as films on liquid. (Schanderl, 1936, prefers to call the film stage of the flor yeasts, the 'oxydative stage', thus emphasizing the importance of oxygen in their metabolism). In the laboratory, satisfactory conditions for growth are provided in glass jars, bottles or flasks closed with corks or rubber bungs, through which holes have been drilled. These holes are loosely plugged with cotton-wool to keep out contaminating organisms, and the flor obtains its supply

of oxygen from the air which diffuses through the pierced corks. Attempts to grow the flor film on wines in sealed containers have always been unsuccessful unless the volume of the headspace is very large in comparison to the area of the film.

In an experiment, different amounts of wine were placed in a number of 4-oz. bottles and seeded with flor culture. The bottles were then closed with waxed corks and sealed with vaspar (equal proportions of vaseline and paraffin wax), and incubated at 20° C. The area of the surface of wine in each bottle was approximately 14 square centimeters, and the volumes of wine, headspace and extent of film growth were as follows:

1. Bottles containing 10 ml. of wine and 108 ml. of air. Complete film cover occurred in about two weeks, but films remained thin and had become dark brown in colour by the fourth week.
2. Bottles containing 25 ml. of wine and 93 ml. of air. Growth and appearance of the films in these were very similar to the previous group, but films were a little thinner.
3. Bottles containing 50 ml. of wine and 68 ml. of air. In these bottles the films never covered more than about 80 per cent. of the surface of the wine and they were thin and dark in colour.
4. Bottles containing 75 ml. of wine and 43 ml. of air. In these bottles film growth was further reduced and never covered more than about 40 per cent. of the surface.
5. Bottles containing 100 ml. of wine and 18 ml. of air. Only very little growth occurred in these, and never covered more than about 10 per cent. of the surface.

In this experiment, even the bottles with the largest headspace did not provide enough oxygen to satisfy the needs of the film.

When growing vigorously the flor film is able to consume the oxygen in the headspace of the culture vessel rapidly.

Some measure of the effects of different oxygen tensions and of the rate at which oxygen is consumed by the film have been obtained from controlled aeration experiments in the following way. Wine was seeded with flor film in a number of large (32-oz.) culture bottles and incubated at 20° C. When the films had just covered the surface of the wine but were still thin, the wine was carefully drawn off from below the film in each bottle and fresh wine added so as to refloat the films with little damage to them. The bottles were then closed with rubber bungs fitted with suitable gas inlet and outlet tubes and sampling tubes. The culture bottles were then joined together in series so that a stream of air could be passed continuously through the headspaces at a controlled rate. Since the flor film consumes oxygen and gives off carbon dioxide, it was possible by adjusting the rate of air flow and the number of cultures in the series, to obtain a gradient in the oxygen content of the headspace gas throughout the series. The percentages of oxygen and carbon dioxide above each culture were determined by analysis at intervals. Owing to fluctuations in the activity of the culture it was not found practicable to maintain the oxygen content of the gas in each culture bottle constant for any considerable period, but the following observations were made.

The rate of film growth was greatest in the cultures receiving the most oxygen, but was not greatly retarded by reduced oxygen content down to about 10 per cent. Film development was considerably retarded, however, with lesser amounts of oxygen and was very slight when the oxygen content of the gas in the headspace fell below about 2 per cent.

In Table 8 are shown the percentage of oxygen in the headspace gas, together with the total aldehyde and volatile acidity of the wine in cultures arranged in the manner described above.

From these figures it can be seen that aldehyde accumulation was greatest in the cultures receiving the most oxygen. When the oxygen content of the gas in the headspace fell below about 1 per cent., as it did in cultures 3, 4 and 5 in both of the above experiments, the aldehyde content of the

TABLE 8

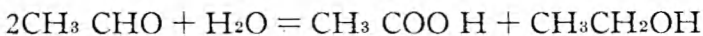
Effect of oxygen supply on aldehyde and volatile acid content of laboratory cultures.

Time in days	Culture 1			Culture 2			Culture 3			Culture 4			Culture 5		
	O ₂ %	Alde- hyde	Vol. acid	O ₂ %	Alde- hyde	Vol. acid	O ₂ %	Alde- hyde	Vol. acid	O ₂ %	Alde- hyde	Vol. acid	O ₂ %	Alde- hyde	Vol. acid
	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.	p.p.m. gm./l.
Exp. I															
0		128	·33		128	·33		128	·33		128	·33		128	·33
4	18·0			17·6			15·7			13·7			11·5		
7	17·3	213		15·3	230		13·6	234		9·1	228		8·2	200	
14	13·2	251	·22	8·2	250	·22	3·5	242	·23	0·9	210	·23	0·7	180	·22
21	9·1	322	·16	3·1	270	·22	0·9	230	·23	0·6	144	·23	0·4	119	·27
28	11·5	319	·15	5·1	289	·15	1·2	177	·17	0·6	129	·19	0·6	120	·25
32	13·1			5·4			0·9			0·8			0·6		
35	15·9	467	·87	5·4	386	·17	0·7	194	·15	0·3	123	·15	0·2	105	·25
40	18·4			11·0			4·0			0·9			0·3		
42		304	1·95		458	1·25		324	·71		178	·13		166	·16
49	19·2	323		18·9	343		14·9	452		8·5	203		4·2	234	
Exp. II															
0		128	·33		128	·33		128	·33		128	·33		128	·33
7	14·9	252	·23	12·3	193	·25	9·0	230	·28	5·7	195	·28	1·8	150	·28
14	11·1	311	·22	6·7	216	·25	2·7	246	·30	0·9	190	·28	0·7	130	·28
21	8·7	369	·15	3·9	241	·23	1·0	232	·23	0·6	150	·23	0·4	100	·28
28	11·5	429	·20	3·6	301	·17	0·8	212	·20	0·7	129	·23	0·4	92	·30
35		405	1·65		478	·59		255	·15		161	·17		153	·20
40	19·7			16·9			8·3			3·5			1·2		
42		316		480				340			245			196	

wine actually decreased instead of increasing. The aldehyde increased again, however, when the oxygen content of the headspace rose.

The results of analyses of the headspace gases in a large number of laboratory cultures and in casks in wineries, have indicated that wines do not increase in aldehyde content under the influence of flor films when the oxygen content of the atmosphere above the film falls below about 1 per cent. This figure was obtained by the analysis of gas samples drawn from the headspaces of casks and laboratory cultures, however, and, since the cells in the flor film consume oxygen and give off carbon dioxide, it is reasonable to suppose that the atmosphere in immediate contact with the films contained rather less oxygen than the samples which were analysed.

Further evidence of the dependence of aldehyde accumulation on oxygen supply has been obtained from experiments with suspensions of flor yeast cells. When the suspension is shaken with 14 per cent. alcohol under an atmosphere of ordinary air, aldehyde accumulates to the extent of between 0.4 and 0.6 mgrm. per ml. in three hours, but when the experiment is carried out under an atmosphere of pure nitrogen, that is, under anaerobic conditions, aldehyde does not accumulate. In fact, if aldehyde is added to the mixture before it is shaken under anaerobic conditions, it is found that the added aldehyde disappears during the experiment and acetic acid is formed in amounts equivalent to slightly less than half of the aldehyde consumed. It seems that under anaerobic conditions the flor yeast converts acetaldehyde to alcohol and acetic acid according to the following equation:



When the experiment was carried out under atmospheres of air diluted with different proportions of nitrogen, it was found that aldehyde accumulation was not greatly depressed unless the oxygen content of the atmosphere was lowered very considerably. The results of such an experiment are shown in Table 9. It can be seen that accumulation of aldehyde was considerably decreased at low oxygen tensions.

TABLE 9

Effect of oxygen on aldehyde accumulation

200 mgrm. flor yeast cells shaken three hours in 14 per cent. alcohol at 18° C. Atmosphere different mixtures of air and nitrogen, pH buffered at 3.25 with M/20 phosphate, total volume of liquid 10 ml. per vessel.

Vessel No.	Atmosphere	Per cent. oxygen	Aldehyde mgrm./ml.
1. }	air	21	·455
2. }		21	·437
3. }	1 vol air +	7	·406
4. }	2 vols. nitrogen	7	·413
5. }	1 vol. air +	3.5	·367
6. }	5 vols. nitrogen	3.5	·368
7. }	1 vol. air +	0.1	·120
8. }	215 vols. nitrogen	0.1	·134
9. }	nitrogen		·015
10. }			·019

(2) Concentration of carbon dioxide in the headspace

Because the film consumes oxygen and gives off carbon dioxide, the concentration of the latter gas in the atmosphere above the film always increases as the oxygen content decreases. In casks simply ventilated by holes in the bungs, the carbon dioxide content of the atmosphere above the films has been found to rise to as high as 20 per cent. when the oxygen had been almost exhausted. However, in casks and culture bottles ventilated by a continuous stream of air through the headspace, the carbon dioxide content has not risen above 15 per cent. even when the oxygen has fallen below one per cent. Because of the simultaneous increase in carbon dioxide and decrease in oxygen, the effects of these two factors are always superimposed under winery conditions, but it is of interest to separate them for experimental purposes. In order to make such a distinction air diluted with various proportions of carbon dioxide has been passed continuously through the headspaces of culture bottles containing growing cultures. The effects of this treatment on the growth of the films and aldehyde accumulation have

been studied and compared with the results of the experiments outlined in the previous section.

In Table 10 are shown the effects of different mixtures of air and carbon dioxide on film growth. In this experiment, ventilation of the cultures with the appropriate gas mixtures was commenced immediately after inoculation. It can be seen that a concentration of 20 per cent. of carbon dioxide, which is equal to the highest found above the film in a cask, retarded film development only slightly, so that the effect of poor ventilation on film growth under normal winery conditions, must be ascribed to lack of oxygen rather than to excess of carbon dioxide. On the other hand, we see from Table 10 that high concentrations of carbon dioxide (50 per cent. and over) retarded film growth very markedly, for the data from previous experiments indicate that the poor growth in these cultures cannot be accounted for by lack of oxygen.

TABLE 10

Effect of carbon dioxide concentration on growth of flor on wine

Gas Mixture	Film growth as percentage cover	
	After 21 days	After 28 days
Air	75	100
80% air + 20% CO ₂	60	90
50% air + 50% CO ₂	25	30
20% air + 80% CO ₂	15	15
10% air + 90% CO ₂	10	10

In Table 11 we see the influence of various mixtures of air and carbon dioxide on aldehyde accumulation by the film growing on wine. This experiment resembled the previous one except that the controlled ventilation started on cultures which had just covered the surface of the wine and had been selected for uniform appearance and aldehyde content.

It is evident that the accumulation of carbon dioxide (up to 20 per cent.) in casks cannot reasonably be blamed for lack of aldehyde accumulation when ventilation is poor under industrial conditions. The experiments show that provided there is sufficient oxygen present the addition of 20 per cent.

of carbon dioxide actually favours aldehyde accumulation rather than depresses it. At high concentrations of carbon dioxide this effect is still more in evidence and some laboratory cultures have reached over 800 mgrm. per litre of aldehyde under an atmosphere of 80 per cent. carbon dioxide and 20 per cent. air.

TABLE 11

Effect of carbon dioxide concentration on aldehyde accumulation

Culture No.	Aldehyde as mgrm./litre acetaldehyde			
	1	2	3	4
Gas mixture	Air	80% air + 20% CO ₂	50% air + 50% CO ₂	20% air + 80% CO ₂
Time in days				
0	204	216	199	211
2	285	290	308	318
4	372	377	428	398
6	398	413	520	520
8	408	460	600	612
10	398	495	694	699
13	403	505	744	770
16	352	444	699	734
18	372	433	678	683

Our evidence on the influence of the composition of the atmosphere above the film may thus be briefly summarized in this way.

A low oxygen content and medium carbon dioxide content inhibits growth and depresses aldehyde accumulation (Table 8).

A low oxygen content depresses aldehyde accumulation in the absence of carbon dioxide (Table 9).

Increasing the carbon dioxide concentration has but little effect on film growth until we reach levels higher than 20 per cent., the maximum normally found in casks of wine carrying flor films (Table 10).

Increasing the carbon dioxide concentration, far from impeding aldehyde accumulation, actually favours it, but here again the effect is probably not significant at the levels of carbon dioxide which normally occur in sherry casks (Table 11).

It seems, therefore, that the effects of inadequate ventilation of the headspace, both in retarding film growth and in depressing flor character development, are primarily due to restriction of oxygen supply.

(3) *Temperature*

Temperature has a very important effect on film development and, in accordance with the data presented by Hansen for other *Saccharomyces* nearly 70 years ago, we find that the temperature range for film formation by the flor yeasts is narrower than the range for the fermenting phase of the same organisms. In grape juice, the flor yeasts are able to grow and bring about fermentation at all temperatures from about 5° C. to a little over 40° C. (41 to 104° F.), although the range for satisfactory fermentation is very much narrower. Film formation on wines occurs only within a much narrower range of temperatures, however. The upper limit of this range varies somewhat with the strain of flor, with the alcohol content of the wine, and probably with other factors, but on wines containing between 14 and 15 per cent. of alcohol by volume (24.5 to 26.2 per cent. P.S.) the upper limit for complete film formation seems to be between 23 and 27° C. (73.4 and 80.6° F.) for most strains. At higher temperatures film growth, if it occurs at all, is restricted to small, thin patches which are usually dark in colour and even completely grown films become dark and sink in the wine if subjected to high temperatures for any considerable time as sometimes happens during the late summer under winery conditions. Most rapid growth on wines occurs at temperatures near 20° C. (68° F.) with all strains so far tested. At temperatures below about 15° C. (59° F.) film formation on wines is usually very slow, although most strains are capable of forming films at temperatures down to about 10° C. (50° F.).

The effects of different temperatures on film growth are shown in Table 12, which lists the times taken by four strains to form complete films on a dry white wine containing 14.6 per cent. of alcohol (25.6 per cent. P.S.) at different temperatures in the laboratory. Four cultures of each yeast were incubated at each temperature.

TABLE 12

Effect of temperature on film growth by four strains of flor on wine

Results in days taken to form complete films.

Strain	15° C.	20° C.	25° C.	30° C.
J4	23-27	16-18	trace	No growth
J12	26-28	15-17	23-25	No growth
J19	25-28	14-16	26-28	No growth
J21	24-28	15-17	trace	No growth

As can be seen from the table, none of the strains grew as films at 30° C. At 25° C. strains J4 and J21 produced only a few small islets dark-brown in colour, and although J12 and J19 produced complete films these were dark in colour and thin as compared with the films produced at the lower temperatures. Moreover, the films grown at 25° C. became moist, broke and sank in the wine about six weeks after they had become complete, whereas those grown at 15° C. and 20° C. were still quite stable when the cultures were discarded after fourteen weeks. Aldehyde determinations were carried out in this experiment only at the seventh and thirteenth weeks, which was not often enough to allow the course of aldehyde accumulation at different temperatures to be compared in any detail. The analyses did show considerable accumulation of aldehyde by all strains at both 15° C. and 20° C., however. No accumulation of aldehyde was detected in cultures incubated at 30° C., while at 25° C. small increases in aldehyde content were noticed with strains J12 and J19, but not with J4 or J21.

The influence of temperature on the changes which the flor brings about in several other wines has been studied in some detail with laboratory cultures incubated at 15° C. and 20° C. The changes occurring in these have been followed by means of frequent determinations of aldehyde content, redox potential, volatile acidity and occasional determinations of alcohol, volatile esters, glycerol and reducing sugar. Samples of the wines have also been submitted to independent tasters for comment. In all these experiments film growth has been more rapid and the films have been heavier at 20° C. than at 15° C. Likewise, the rates of consumption

of alcohol, sugar and glycerol have been greater at 20° C. than at 15° C.

The changes in redox potential of the cultures also differ according to the temperature of incubation. At both temperatures the potentials fall, and subsequently rise again, but they fall more rapidly and usually reach lower levels at 20° C. than at 15° C. The volatile acidity of the wine also falls more rapidly at 20° C. Volatile ester production at the two temperatures has not differed greatly in the wines examined, but in most of them it has been slightly greater at 15° C.

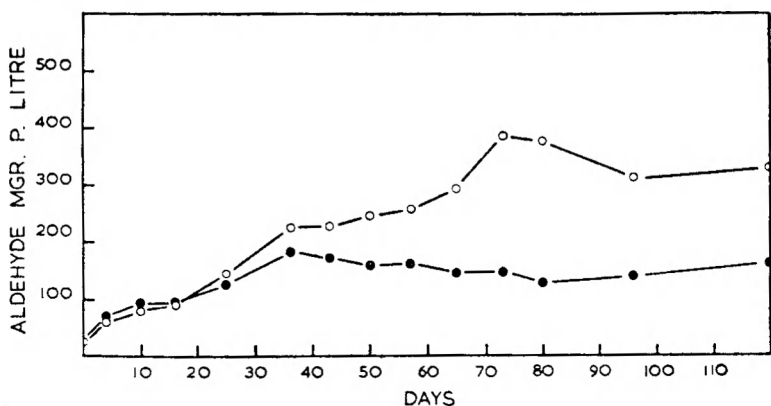


Fig. 10. Course of aldehyde accumulation in a 1949 Palomino at two different temperatures.

○ ——— ○ 15° C.; ● ——— ● 20° C.

As far as the results of chemical analyses are concerned, the most interesting differences between the effects of incubating at 15° C. and 20° C. are seen in the accumulation of aldehyde at these temperatures. We find that in some wines aldehyde accumulates more regularly and reaches high values earlier at 15° C. than at 20° C., while in other wines the rate of accumulation is appreciably greater at 20° C. than at 15° C. The curves shown in Figs. 10 and 11 illustrate typical behaviour of both types of wines. The effect of temperature on aldehyde accumulation is thus found to depend on the composition of the particular wine. In general it has been observed that wines on which film

development is very vigorous, behave like the one represented by Fig. 10 in accumulating aldehyde more regularly at the lower temperature. On the other hand, the wines which behave like the one represented by Fig. 11 are usually those on which film development is only moderately vigorous.

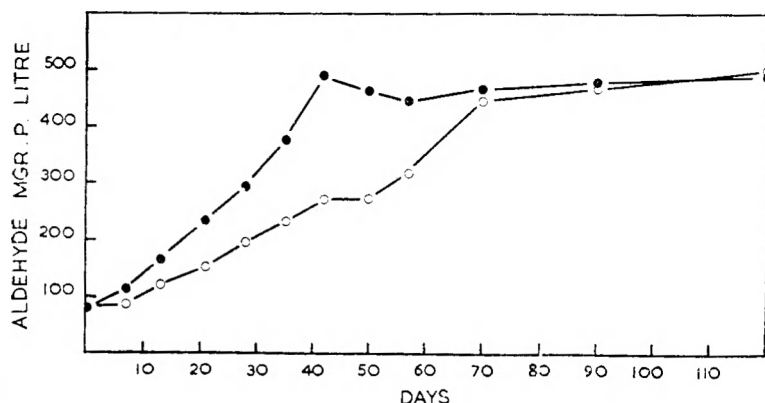


Fig. 11. Course of aldehyde accumulation in a 1948 Pedro at two different temperatures.

○ ——— ○ 15° C.; ● ——— ● 20° C.

4. THE COMPOSITION OF THE WINE

Although some of the variations noticed in growth of the film and development of flor character are undoubtedly due to differences between strains of flor and between environmental conditions, other variations are certainly due to differences between the wines themselves. Thus, even under carefully controlled conditions in the laboratory it is often found that two wines respond quite differently when seeded with the same strain of flor and incubated under conditions which are as nearly identical as possible. Some of the differences noticed in the effects of the flor on different wines were mentioned in the last chapter and illustrated in Figs. 1, 3, 4 and 5. In this section the influence of the composition of the wine on film growth and flor character development, particularly aldehyde accumulation, will be considered in more detail.

(1) *The alcohol content of the wine*

Ethyl alcohol is one of the factors which are essential for vigorous growth of the flor film. In media without alcohol only weak film development and no significant accumulation of aldehyde has occurred. However, although necessary for film growth and aldehyde accumulation, alcohol has germicidal properties, and so we find that the concentration of this substance has a very profound effect on the growth and metabolism of the flor on wine or artificial medium.

As might be expected, the lower the alcohol content of wine within normal limits, the more rapid is film growth, but the effect of a particular concentration of alcohol on film growth is influenced by several other factors, notably the temperature, the strain of flor used and its cultural history. During these investigations some strains have failed to form complete films on wines containing 12 per cent. or more of alcohol (21 per cent. P.S.), and although they have grown well at lower strengths, are consequently quite unsuitable for making sherry. Other strains have grown complete films on wines containing 16.5 per cent. of alcohol (28.9 per cent. P.S.) and partial films on 17 per cent. alcohol (29.8 per cent. P.S.) but growth has always been very slow at strengths above about 15.5 per cent. of alcohol (27.1 per cent. P.S.) At strengths near the maximum tolerated by the particular strain, the films not only grow more slowly, but also remain thinner and smoother than at lower strengths.

No significant differences have been noticed between the maximum amounts of alcohol tolerated at 15° C. and at 20° C. although growth is always more rapid at the latter temperature. Above this temperature, however, the alcohol tolerance of the film falls off quite markedly, so that at 25° C. many strains fail to form complete films on wines containing more than about 12 per cent. of alcohol (21 per cent. P.S.) although they grow well on 14 per cent. at lower temperatures.

The extent to which the alcohol tolerance of the film stage of the flor yeasts can be raised by habituation, has not been systematically investigated, but it is commonly found that

this tolerance is influenced by the conditions under which the particular strain has recently been grown. For example, a flor yeast transferred from an old agar culture does not as a rule grow readily as a film on wine of normal alcohol content until the organism has had some habituation. This is most readily achieved by allowing it to ferment some grape juice and subsequently form a film on the fermented liquid. This film may then be used for inoculating wine.

It is of some interest to compare the tolerance to alcohol of the strains of flor studied here with the results reported by other workers. Marcilla (1939) found that the optimum alcohol concentration for film formation by a typical Spanish strain was between 13.5 and 14.5 per cent. by volume (23.6 and 25.4 per cent. P.S.) while the maximum for complete films was 16.5 to 17 per cent by volume (28.9 to 29.8 per cent. P.S.) and for partial films 17.3 to 17.5 per cent. (30.3 to 30.6 per cent. P.S.). Cruess (1948) found the maximum alcohol content of wines on which his strains formed films was between 16 and 17 per cent. (28 and 29.8 per cent. P.S.) We see then that several of the strains in use in Australia resemble those described by overseas workers quite closely in their tolerance to alcohol, but here we have not found an optimum alcohol content for film growth as high as that reported by Marcilla. Our experience has been that the rate of film growth is increased by lowering the alcohol content of the wine or other medium at least down to three or four per cent. although films grown at such low strengths are commonly very unstable and break and sink in the medium early.

Some of the effects of alcoholic strength on the changes brought about in a wine by the flor film are shown in Table 13. The wine used in this experiment was divided into three lots, the alcohol contents of which were adjusted to 14.5, 15.2 and 16.0 per cent. by volume respectively (25.4, 26.6 and 28.0 per cent. P.S.) by appropriate additions of rectified grape spirit. Each lot of wine was then divided into five 800 ml. portions in 32-oz. culture bottles making fifteen in all. Three bottles of each wine were then inoculated with flor yeast and the other two kept as un-

inoculated controls to indicate the loss of alcohol by evaporation. All bottles were incubated at 20° C. The alcohol content of each culture was determined by the dichromate oxidation method and readjusted to its initial value each week. The alcohol lost from cultures in excess of that lost from uninoculated controls was reckoned as alcohol consumed by the flor, it being assumed that evaporation losses from cultures and controls were similar. Actually, however, this is only an approximation, for the presence of the flor film on the surface of cultures is likely to have had some effect on the evaporation from these.

The results presented in Table 13 represent the analysis at the end of thirteen weeks of incubation. Earlier analyses showed that the volatile acidity fell to about 0.2 grm. per litre in all cultures, and later rose again in all except those with 16 per cent. alcohol. From these results it appears that increasing the alcohol content of the wine from 14.5 to 15.2 per cent. (25.4 to 26.6 per cent. P.S.) has had very little effect on either the growth of the film or the changes brought about in a wine. However, when the strength was raised to 16 per cent. alcohol (28 per cent. P.S.) both film growth and metabolism were noticeably retarded.

Actually the effect of alcoholic strength on aldehyde accumulation can be seen much better if we examine the course of aldehyde accumulation throughout the experiment as shown in Fig 12 rather than the final analysis. Here we notice that increasing the alcohol content of the wine had no effect on the accumulation of aldehyde during the first three weeks of incubation, but thereafter the higher alcohol levels depressed aldehyde accumulation very considerably. The depression of aldehyde accumulation by these amounts of alcohol seems to be confined to the completed films. In this experiment no such effect was evident during the first three weeks, while the film was still incomplete. In other experiments, wines containing up to 16.5 per cent. of alcohol (28.9 per cent. P.S.) have increased in aldehyde content by over 170 mgrm. per litre during the early period of active film growth. Moreover, experiments with suspensions of flor yeast cells shaken in various

concentrations of alcohol have shown that appreciable amounts of aldehyde are formed even in 25 per cent. alcohol (43·8 per cent. P.S.) and that over a period of three hours the greatest aldehyde accumulation occurs in about 18 per cent. alcohol (31·5 per cent. P.S.). We see, therefore, that the effect of a particular concentration of alcohol on the accumulation of aldehyde by the flor yeast, depends on other circumstances. Actually, in cellar practice the alcohol content of flor sherry material is governed largely by considerations of safety—safety from attack by acetic acid

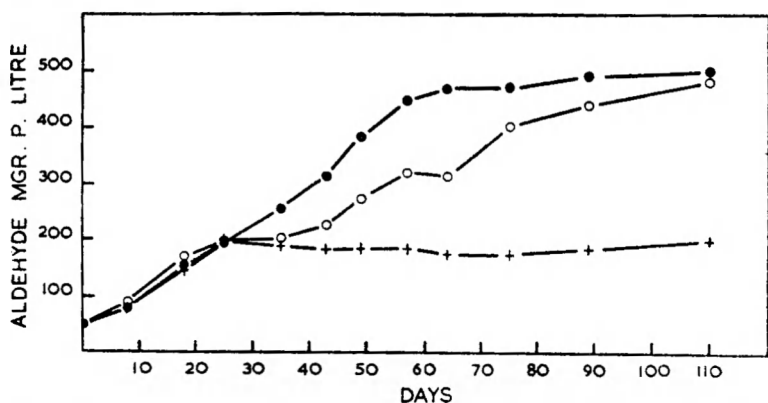


Fig. 12. Course of aldehyde accumulation in a 1948 Pedro with alcohol content adjusted to different levels.

- ——— ● 14.5 per cent. alcohol (25.4 per cent. P.S.)
- ——— ○ 15.2 per cent. alcohol (26.6 per cent. P.S.)
- + ——— + 16.0 per cent. alcohol (28.0 per cent. P.S.)

bacteria—and for this reason it is important that it should not be below about 14.5 per cent. by volume (25.4 per cent. P.S.). Although a slight increase above this figure may be considered desirable to increase the margin of safety, experimental results indicate that higher strengths do not improve flor character development but rather the reverse if the alcohol is raised above about 15 to 15.5 per cent. (26.2 to 27.1 per cent. P.S.).

(2) *Sugars and other carbon compounds in wine which may serve as nutrients for the flor film*

When compounding artificial media for the flor it has been found necessary to include another carbon source as well as ethyl alcohol in order to obtain vigorous film growth. Of the possible carbon sources tested, glycerol has given most vigorous growth with lactate almost as good. Small amounts of glucose or fructose, up to about 1 grm. per litre, stimulate growth, but larger amounts usually delay the appearance of film until the sugar has been fermented. Acetic, tartaric, malic and citric acids, when added to artificial medium, all supported some film growth, but less than glycerol, lactate, glucose or fructose.

Because of the rather wide variations found in the sugar content of wines intended for flor sherry some attention has been given to the effects of such variations on growth of the flor and development of flor character. The wines commonly used as flor sherry material in this country have been found to contain between 0.8 and 1.2 grm. per litre of non-fermentable sugar, probably chiefly arabinose, which is not used by the flor film. In addition to this there are variable amounts, from less than 0.2 grm. up to 2 or even 3 grm. per litre, of fermentable sugar which is almost completely consumed by the flor. Although the addition of small amounts of sugar has stimulated film growth on some experimental media, we have not, during these investigations, encountered a wine on which film growth was significantly stimulated or aldehyde accumulation increased by the addition of sugar or of grape juice. When the amounts added have been less than about 1 grm. of sugar per litre, they have usually not had any noticeable effect. Amounts exceeding about 5 grm. per litre have always promoted visible fermentation and have delayed film growth by several weeks while intermediate amounts have often retarded film growth but have sometimes been without effect. Usually films are not formed on wines which contain more than a few grms. per litre of fermentable sugar, but when such wines are inoculated, the flor yeast often grows as a deposit at first and ferments the excess sugar. Because

of this fermentation, the headspace above the wine becomes charged with carbon dioxide, and this in itself is enough to prevent film growth until the excess sugar has been fermented. The fermentation of this sugar may be very slow under winery conditions so that the headspace is filled with carbon dioxide over a period of several weeks or even months if ventilation is too restricted and film growth is correspondingly delayed. In some cases when wines are inoculated by carefully floating portions of film on the surface, a complete film may be formed even though the wine contains several grms. of fermentable sugar per litre. Sooner or later, however, cells falling from the film growing on such a wine commence growing in the depth of the wine and set up a fermentation of the residual sugar. It is not uncommon to find a film raised in places by bubbles of entrapped carbon dioxide arising from such a fermentation. Usually the effect is slight and only transitory, but occasionally the fermentation is vigorous enough to break up the film or to raise the concentration of carbon dioxide in the headspace sufficiently to check the growth of the film for several months. Under these latter conditions the development of flor character is much delayed, but experiments have indicated that the presence of smaller amounts of fermentable sugar (1.5 to 2.0 gm. per litre) which do not promote visible fermentation, may also delay the development of flor character. It has been observed that wines with a high residual sugar content usually develop flor character poorly or very slowly even when the amount of sugar is not sufficient to check film growth very much. The addition of sugar to wines either as grape juice or as glucose or fructose, has retarded aldehyde accumulation to a varying extent, depending on circumstances. The curves in Fig. 13 represent the accumulation of aldehyde in a 1948 Pedro in laboratory cultures after 0.6 per cent. of sterile grape juice (equivalent to 1.5 gm. per litre of invert sugar) had been added to the wine. The two curves in this figure correspond to two incubation temperatures, 15° and 20° C. Comparison of these curves with those for the same wine without any additions (Fig. 11) shows the influence of the grape juice

on the accumulation of aldehyde. It can be seen that the accumulation of aldehyde has been depressed by the grape juice at both temperatures but the effect has been much more prolonged at 15° than at 20° C. This effect on the aldehyde has always been accompanied by a corresponding effect on the oxidation-reduction potential of the wine. The fall in potential, which is one of the characteristic changes occurring in wines during the growth of flor, is always greater and more prolonged in the wines to which sugar or grape juice has been added.

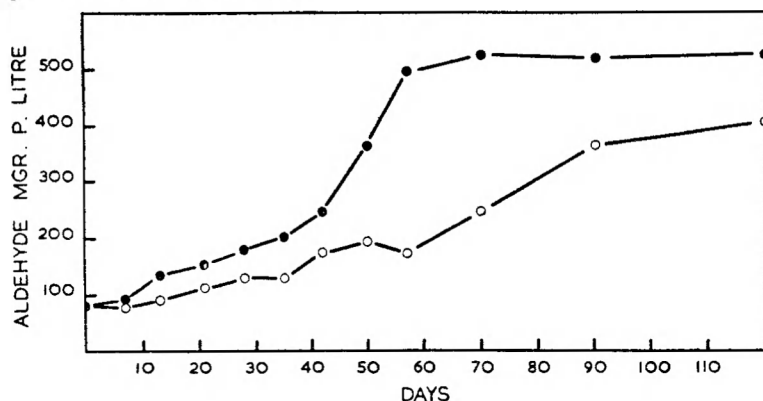


Fig. 13. Course of aldehyde accumulation in 1948 Pedro with 0.6 per cent. grape juice added and incubated at two temperatures.

○ ——— ○ 15° C.; ● ——— ● 20° C.

In addition to laboratory experiments in which sugar or grape juice has been added to wines, the course of aldehyde accumulation has been followed in many wines containing different amounts of sugar under winery conditions. The evidence available from these sources all indicates that wines which have a high content of residual sugar are not the most suitable for flor sherry. Likewise, increasing the sugar content of wine by the addition of either sugar or grape juice does not favour flor growth nor the development of flor character but retards them.

As already pointed out, glycerol and some of the organic acids of wine can also be utilized by the flor film. In artificial culture media not containing other suitable carbon

sources, the omission or inclusion of glycerol, lactate, tartrate, malate or citrate has considerable influence on growth of the flor and the accumulation of aldehyde, but normal variations in the amounts of these substances in wines have not been found to have any such influence.

The effect of acetic acid on the flor yeast is worthy of special mention because the ability of the flor film to grow on wines of high volatile acidity and to destroy this acid has often been mentioned. Cruess and Podgorny (1937) grew flor on wine which initially contained 2.58 gm. per litre of acetic acid and found that this had been reduced to 0.53 gm. per litre after 46 days. Saenko (1945) obtained slow but complete film growth on wines containing up to 4.0 gm. per litre of acetic acid and found that this had fallen to 1.35 gm. per litre after 45 days.

In our own investigations no attempt has been made to determine the highest volatile acidity tolerated by the flor, for this figure would vary with the pH of the wine and other factors. The flor film has been grown in the laboratory on wines, with a pH of 3.1 containing 14.5 per cent. of alcohol (25.4 per cent. P.S.) and up to 2 gm. per litre of acetic acid. The volatile acidity of such wines always fell quite rapidly, usually to below 0.4 gm. per litre. Adjusting the volatile acidity of wines by adding acetic acid has had very little effect on film growth until the total volatile acid exceeded about 1 gm. per litre, but higher doses have retarded growth appreciably. The development of flor character has been retarded somewhat when growth was retarded, but small additions of acetic acid to the wine have accelerated aldehyde accumulation slightly in some wines and have had no effect in the case of others.

The effect of acetic acid on flor growth and aldehyde accumulation is probably of little or no consequence in normal flor sherry material since these wines initially contain less than 1 gm. per litre of acetic acid and this value is further reduced by the film itself when it grows. The retarding effect of acetic acid may become important, however, if attempts are made to improve or reclaim 'pricked' wines by means of the flor film, as has been recommended by various

authorities (Cruess, 1937; Saenko, 1945; Bobadilla, 1943). If the volatile acidity of such wines is high, film growth may be so slow that special precautions must be taken to maintain both the alcohol and the SO_2 at levels high enough to prevent acetic acid bacteria growing on the surface again before the flor forms a complete cover.

(3) *The pH value of the wine*

Marcilla (1939) found that a typical strain of flor grew most rapidly when the pH of the wine was about 3.2, while the minimum and maximum values for growth on wine were about 2.7 and 4.2 respectively. Cruess (1948) using wines with a pH range of 2.65 to 3.95, obtained film growth over the whole of this range, but growth was definitely retarded below pH 3.0.

In our own investigations the extreme limits of the pH range over which the flor film is able to grow on wines, have not been determined, but a number of experiments have been carried out to determine the effects of different pH values over a range which is wider than that found in the values of wines used as sherry material. Complete films have been grown on wines with pH values adjusted to various levels over the range 2.8 to 4.8.

Although the effect of pH on film growth varies from one wine to another, growth is usually slow and the films remain thin when grown on wines with pH values below about 3.0. At pH 3.0 the films usually take longer to cover the surface of the wine than at 3.2, but after some months there may be little difference in the appearance of the films grown at these two pH values. Raising the pH of the wine from 3.2 up to about 3.5 usually has little or no effect on the rate of growth of the flor, but there are some wines on which the film becomes very heavy, moist and unstable if the pH is raised above the latter value. Such films break and sink in the wine relatively early, flor character development is unsatisfactory and the wines commonly acquire a yeast flavour.

In practice, it is desirable that the pH of sherry material should be between 3.1 and 3.4. Wines with values below

this range not only grow the flor slowly, but are usually too sour to be palatable. On the other hand, if the pH value is much above this range, flor character development is often unsatisfactory and, moreover, the danger of spoilage by lactic acid bacteria is greatly increased. It is indeed a fortunate circumstance for the maker of sherry that the range between pH 3.1 and 3.4 is very favourable for flor, and it is thus possible to keep the pH of wines low enough to prevent lactic acid spoilage without inhibiting the growth of the flor or development of flor character.

(4) *The nitrogen content of the wine and the addition of various nitrogen sources*

No attempt has been made to carry out a survey of the nitrogen content of the wines used as sherry material in Australia, but from the results of analyses which have been carried out, it seems likely that most of these wines contain between 100 and 200 p.p.m. of nitrogen. Thus out of sixteen wines analysed only two contained less than 100 p.p.m. and two contained more than 200 p.p.m. of total nitrogen, the lowest and highest figures being 66 and 250 p.p.m. respectively. It has not been found that the total amount of nitrogen present gives a reliable indication of the suitability of a wine for flor growth, nor indeed is such a relationship to be expected, for the form in which the nitrogen occurs is just as important as the amount present. Although the various fractions of nitrogen occurring in the wines under discussion have not been determined, it has been found that most wines give some increase in film growth after the addition of nitrogen in a form readily used by the flor, such as diammonium phosphate or ammonium sulphate. The response to such additions varies considerably from one wine to another, however. Most of the wines studied, even those containing as little as 100 p.p.m. of nitrogen, have grown satisfactory films without additions, but have grown rather heavier films and grown them more rapidly after the addition of 0.2 to 0.5 grm. per litre of ammonium salts. A few wines have produced only very poor growth unless some readily available nitrogen source was added, and one

wine gave a definite response to the addition of as little as 0.12 grm. per litre of diammonium phosphate (25 p.p.m. of nitrogen). On the other hand, some wines which have grown vigorous films without any additions have shown no response to additions of up to 0.5 grm. per litre of ammonium salts.

With all the wines examined, very heavy doses of ammonium salts have produced much the same effect. The film has grown rapidly at first and been pale in colour, but has later become moist and unstable so that sections of it sank to the bottom of the container, leaving portions of the surface uncovered. On some wines a new film has developed, and soon covered the surface again, but on others the new film developed very slowly and remained thin and smooth. The amount of ammonium salts which may be added without causing the film to become unstable varies greatly from one wine to another. One wine originally containing only 66 p.p.m. of nitrogen, grew a heavy but stable film after the addition of 0.75 grm. per litre of diammonium phosphate (about 160 p.p.m. of nitrogen), while other wines receiving half this dose have grown films which became unstable early.

Of the various nitrogenous compounds which have been added to wines during the investigations, ammonium sulphate and diammonium phosphate have given similar results. Urea and asparagine were tested on only three wines, but on these they gave results similar to those obtained with the ammonium salts.

Yeast extract has also been added to a number of wines as a growth stimulant, but this substance is in rather a different category for it serves not only as a source of nitrogen but also as a source of several accessory growth factors required by the flor. The yeast extract used in experiments has been chiefly Difco yeast extract powder which contains about 7 per cent. of nitrogen. It has been found that some wines respond equally well whether nitrogen is added as ammonium salts or as yeast extract, but on others film growth is stimulated much more by yeast extract than by ammonium salts when they are compared in amounts of equivalent

nitrogen content. These latter wines evidently lack something which is required by the flor, other than ammonia nitrogen—this may be accessory growth factors or essential amino acids supplied by the yeast extract. In this connection it is worthy of note that the flor film does not grow vigorously on artificial media containing ammonium salts unless some yeast extract is included.

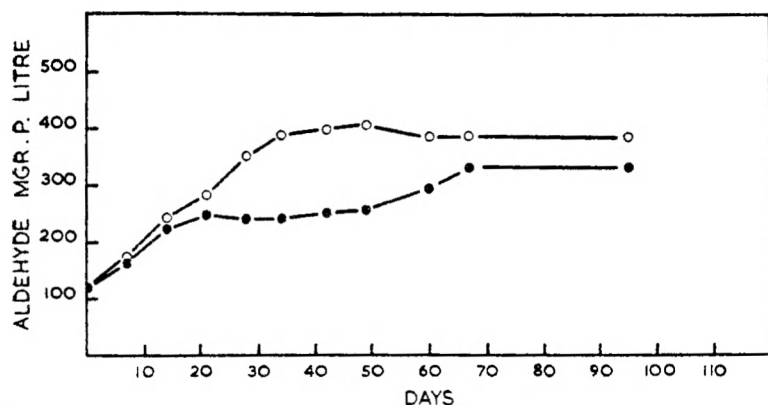


Fig. 14. Course of aldehyde accumulation in a 1945 Pedro with and without ammonium sulphate added. 20°C.

● — ● control.
○ — ○ with 0.05 per cent. $(\text{NH}_4)_2 \text{SO}_4$ added.

The unstable films which grow on wines after the addition of large doses of ammonium salts have already been mentioned. Large doses of yeast extract also lead to very heavy film growth, but these films are usually more stable than those grown on high doses of ammonia nitrogen. It is common to find that clumps of cells are continually being deposited from underneath the films grown on heavy doses of yeast extract. These deposited cells are continually replaced by the growth of new cells so that the film remains thick and heavily wrinkled over the whole surface of the wine for a long period and a heavy deposit of yeast which has dropped from the film, accumulates in the bottom of the container.

Although the addition of small amounts of ammonium salts or other suitable nitrogenous compounds, to wines, usually stimulates the growth of the flor film, the effects on the changes brought about in the wines are very complex. The development of flor character and the accumulation of aldehyde may be increased, decreased or unaltered by the addition of such nitrogenous compounds, depending upon the amounts added, the type of wine and the temperature of incubation. Some of these effects are illustrated by the

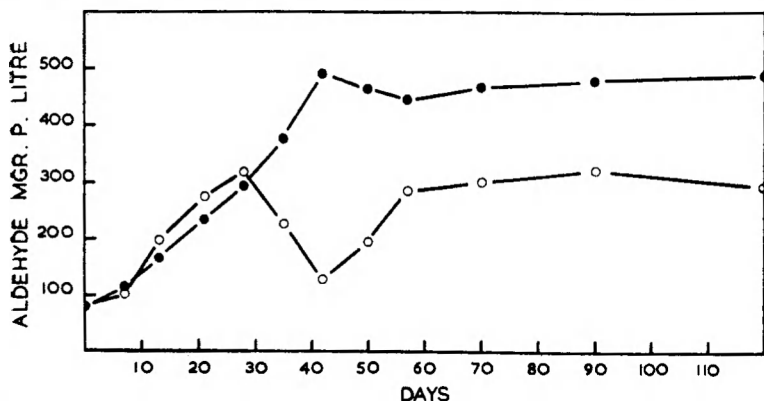


Fig. 15. Course of aldehyde accumulation in a 1948 Pedro with and without ammonium sulphate added. 20° C.

● — ● control.
○ — ○ with 0.05 per cent. $(\text{NH}_4)_2\text{SO}_4$ added.

graphs (Figs. 14, 15 and 16). Fig. 14 shows the effect of adding 0.5 gm. per litre of ammonium sulphate to a 1945 Pedro which originally contained only 66 p.p.m. of total nitrogen. We see that the accumulation of aldehyde was increased by this treatment. However, a similar treatment applied to another wine, this time a 1948 Pedro, affected aldehyde accumulation in the way indicated in Figs. 15 and 16. When the cultures were incubated at 20° C. those which received the extra nitrogen fluctuated in aldehyde content after the fourth week and from then on always contained less aldehyde than the controls. On the other hand, when the cultures were incubated at 15° C. this retard-

ing effect of the ammonium salts on aldehyde accumulation disappeared, and we find the curves for the treated wines and the controls are very similar.

When analyses are compared with the results of observations on film growth, it is found that the relationship between the effects of added nitrogenous compounds on film growth and on aldehyde accumulation may be expressed in the following general terms.

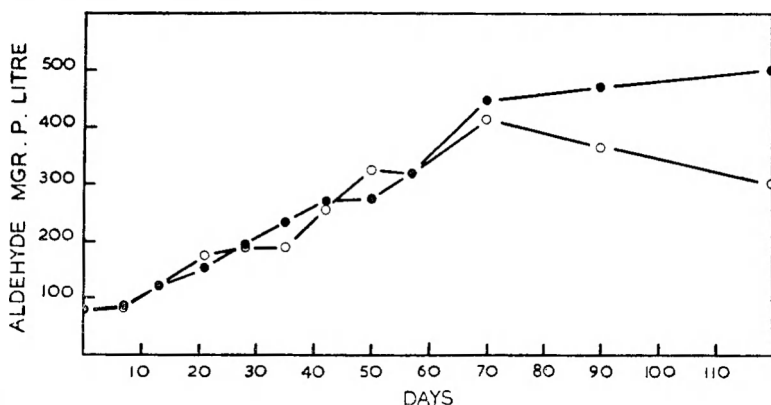


Fig. 16. Course of aldehyde accumulation in 1948 Pedro with and without ammonium sulphate added. 15° C. (c.f. Fig. 15).

- ——— ● control.
○ ——— ○ with 0.05 per cent. $(\text{NH}_4)_2\text{SO}_4$ added.

When growth on the original wine is poor but is stimulated by additions of nitrogenous compounds, then small to moderate additions of such compounds usually increase the accumulation of aldehyde and the development of flor character.

When growth on the original wine is already vigorous, further stimulation of this growth by the addition of nitrogenous compounds depresses the accumulation of aldehyde.

When growth is not affected because of the nature of the wine, because the amount of nitrogen added is very small or because of low temperature, then the accumulation of aldehyde is usually little influenced by the addition.

As regards the influence of added nitrogenous compounds on other changes brought about in the wines, we find that some of these, particularly the consumption of alcohol and glycerol, and the lowering of the redox potential, appear to be very closely related to the vigour of film growth. Thus stimulation of film growth by adding such compounds results in a corresponding stimulation of the consumption of alcohol and glycerol and a greater fall in the redox potential. The fall in volatile acidity, which occurs in wine under flor, is also more pronounced when film growth is stimulated by the addition of available nitrogen, but when very heavy film growth is induced by large doses, and particularly if the substance added is yeast extract, then the volatile acidity of the wine commonly rises again quite markedly after prolonged incubation.

TABLE 14

Effect of yeast extract and ammonium sulphate on some of the changes brought about in wine by the flor film in 14 weeks at 20° C.

	0.66 g./l. ammonium 2.0 g./l. Controls sulphate yeast extract		
Alcohol consumed per cent. by vol.	0.8	1.4	1.7
Glycerol consumed grm./l.	4.0	5.1	6.0
Volatile acid consumed g./l.	.15	.15	.15
Change in pH	— .05	— .20	+ .05
Film growth—days to complete cover	10 to 14 days		
Type of film	mod. heavy	—	very heavy

The effect on the pH of the wine depends on both the type and amount of nitrogenous substance added. When ammonium sulphate or phosphate is added the flor brings about a fall in pH by using the ammonia and leaving sulphuric or phosphoric acid in the wine. Wines which had received 0.5 grm. per litre of these ammonium salts have usually fallen about 0.2 pH units during film growth, but falls of 0.3 have been noticed in some wines. Other nitrogenous substances tested—urea, asparagine and yeast extract—have not affected the pH of the wine unless the amounts

added have been rather large (1 gram. per litre or more) in which case they have caused the pH of the wine to rise slightly.

The effects of ammonium sulphate and yeast extract on some of the changes brought about by the flor in a 1948 Pedro wine in laboratory cultures incubated for fourteen weeks at 20° C. are shown in Table 14.

(5) *The tannin content of the wine*

The tannin content of the wine influences both the rate of film growth and the appearance of the film. In Spain, Marcilla (l.c.) found that the growth of his strains of flor was retarded when the wine contained 0.16 gram. per litre or more of tannin and that with each addition of tannin to a wine in excess of about 0.1 gram. per litre the film became darker in colour.

Observations on the growth of flor on wines to which different amounts of Merck's tannic acid had been added in our laboratory have yielded results rather similar to those of Marcilla. Raising the tannin content of wines to 0.2 gram. per litre has, in most cases, delayed growth only slightly, but the films grown after such additions have always been darker in colour and have usually been thinner and smoother than in control cultures. Higher levels of tannin have always delayed growth and when the amount was raised to 0.4 gram. per litre or more, the films were dark-grey to brown in colour and remained thin and smooth, in some cases becoming moist and submerged in the wine. A few wines have been examined on which growth was apparently retarded by an excess of tannin already present in the wine, for these wines gave better film growth after being fined with gelatine.

No specific effect of tannin on the changes brought about by the flor have been observed. The effect of tannin seems to be that growth is retarded and all the activities of the film are correspondingly depressed. It has been pointed out by many writers, including Castella (l.c.), Marcilla (l.c.) and Cruess (l.c.) that wines containing appreciable amounts of tannin are not suitable for making flor sherry, because they

remain coarse and dark in colour—two very objectionable faults in flor sherry. Our own experience entirely confirms this view.

(6) *The SO₂ content of the wine*

Cruess (1948) found that several strains of flor yeasts brought about vigorous fermentation in less than a week in musts which had initially contained 300 p.p.m. of SO₂. He concluded that the fermenting stage of these yeasts resembled the ordinary wine yeasts (*Saccharomyces ellipsoideus*) in tolerance to SO₂. Marcilla (l.c.) found that his strains were initially rather sensitive and were checked by 130 to 150 p.p.m. in must. However, he was able to habituate them to SO₂ so that they fermented must containing over 200 p.p.m. The strains of flor studied by the two authors mentioned above also differed in their tolerance to SO₂ in the film stage of development. Cruess found that films grew within three weeks on wines whose initial SO₂ content was 250 p.p.m. and within four weeks on those with an initial SO₂ content of 350 p.p.m. Although he did not state the amount of SO₂ present when the films had grown it would certainly have been less than the initial figure, however. As a result of his laboratory and winery experiments Cruess concluded that the film will grow readily on wine in casks containing 180 p.p.m. of SO₂ if the free SO₂ does not exceed 5 per cent. of the total, and he recommended an initial SO₂ content of 150 p.p.m. On the other hand, Marcilla found that an initial dose of 100 p.p.m. of SO₂ delayed film growth for 35 days at 18° C. and that when growth started the level had fallen to 78 p.p.m. However, he was able to grow the flor on wines containing from 180 to 250 p.p.m. if these were inoculated by carefully floating portions of the film on the surface.

Strains of flor in use in Australia have been found to ferment vigorously in musts containing 100 p.p.m. of total SO₂ and no difficulty has been experienced in preparing cultures which have commenced vigorous fermentation within 42 hours when seeded into grape juice to which 350 p.p.m. of SO₂ had been added. Thus in their fermenting stage,

these flor yeasts resemble the ordinary wine yeasts in their tolerance to SO_2 .

However, our strains do not have a high tolerance to SO_2 in the film stage, and in this respect resemble those studied by Marcilla rather than those studied by the Californian workers. In a laboratory experiment, using large jars each containing two litres of wine of pH 3.1 and 14.5 per cent. alcohol (25.4 per cent. P.S.) inoculated with one loop of film and incubated at 20°C ., complete film cover was obtained in four weeks on the controls which received no SO_2 and in five weeks on the wines which had received 100 p.p.m. of SO_2 . However, by this time the SO_2 in these latter had fallen to 70 p.p.m. In another experiment with a similar wine treated with SO_2 at the rates of 150 and 300 p.p.m., the controls without SO_2 had complete films at the end of the fourth week whereas the treated wines showed practically no growth at this stage. Growth started later on these, however, and complete, thin, dark-coloured films were formed by the end of the eighth week on the cultures which had received 150 p.p.m., and by the eleventh week on those which had received 300 p.p.m. These dark films gradually increased in thickness and became light-grey in colour by about the eighteenth week on both series of the sulphited wines. By the time that film growth had covered the wines, the SO_2 content had fallen to between 20 and 40 p.p.m. in all cultures.

In casks of wine, in wineries, growth has been vigorous and normal on wines containing up to 80 p.p.m. of SO_2 when seeded, but doses of over 100 p.p.m. have delayed film growth usually by several weeks. While wines which contained as much as 200 p.p.m. of SO_2 at the time of seeding have eventually developed films, such films have grown only after a long delay when the SO_2 content had fallen considerably—to below 80 p.p.m. in all the wines tested.

Attempts have been made in the laboratory to increase the tolerance of the film stage to SO_2 by habituation, but with very little success. During 12 months of repeated culture on wine with SO_2 added, no marked increase in the tolerance of the film for this substance was observed. Growth

in the presence of 100 p.p.m. appeared to be normal, but with 150 p.p.m. film development was always slow. It was found, however, that if the flor was allowed to produce a complete film on wine, the SO_2 content of this wine could then be raised to over 200 p.p.m. without any noticeable effect on the film.

This seeming higher tolerance towards SO_2 of the established film, as compared with the early stages of film growth, appears to be due to the combination of SO_2 with aldehyde. It has long been known that SO_2 combines with aldehydes to form compounds which are much less toxic than free SO_2 for yeasts. In a series of experiments it was observed that when 200 p.p.m. of SO_2 was added to wine which had carried a flor film and accumulated about 250 p.p.m. of aldehyde, the SO_2 was fixed quite rapidly so that the free SO_2 was only 9 p.p.m. at the end of one hour and had further fallen to below 5 p.p.m. after 18 hours. On the other hand, when other portions of the same wine, which had not carried flor and contained only about 70 p.p.m. of aldehyde, were sulphited at the same rate, the free SO_2 amounted to 70 p.p.m. after one hour and was still 40 p.p.m. after seven days. The total SO_2 in both lots of wine were about the same and remained between 180 and 200 p.p.m. for seven days. Portions of both lots of wine were seeded with flor one hour after the SO_2 was added. It was found that within two weeks films had again grown on the wine which had previously carried flor and so contained little free SO_2 , but only scattered islets of film had grown on the wine which had not previously carried flor and so contained appreciable amounts of free SO_2 .

The difficulty of increasing the tolerance of the film to SO_2 seems to be due to the high toxicity of free SO_2 as compared with fixed SO_2 and to the rapid fixation of SO_2 by aldehyde produced by the flor. Thus an actively growing film used as a parent culture is not growing in the presence of a high concentration of free SO_2 even if the wine originally received quite a high dose. However, when such a film is subcultured into freshly sulphited wine, it is at once exposed to a high concentration of free SO_2 and

growth is very slow until sufficient aldehyde has been produced to fix the SO_2 . In other words, it appears that habituation of the film to SO_2 doesn't readily occur because the free SO_2 doesn't persist in the wine during the growth of the film.

Notwithstanding the lack of success in producing a film culture with a high tolerance to SO_2 , it has been possible to obtain information concerning the effect of SO_2 on aldehyde accumulation and flor character development. Three batches, each of six culture jars, of a wine were treated as follows:

- (a) no SO_2 added; seeded with flor.
- (b) 100 p.p.m. of SO_2 added; seeded with flor 24 hours later.
- (c) 100 p.p.m. of SO_2 added; seeded with flor 24 hours later and a further 100 p.p.m. of SO_2 added after 10 days when the wines were about half-covered with films.

The SO_2 content of each culture was maintained by determinations and appropriate additions at regular intervals.

It was found that film development was slightly retarded by SO_2 at first, but by the end of the third week all cultures had grown complete films and there was little difference in appearance between them.

Aldehyde accumulation was rapid, and by the end of the sixth week exceeded 400 p.p.m. in nearly all cultures. There were no significant differences in aldehyde accumulation due to SO_2 . However, when samples of the wines were tasted it was found that flor character was most evident in the cultures which had not received any SO_2 and was least in those which had received 200 p.p.m. Thus, although the SO_2 did not affect the accumulation of aldehyde under these conditions, it masked the development of flor character.

These results indicate that the flor film is able to tolerate quite high doses of SO_2 once it has produced sufficient aldehyde to fix it, but that SO_2 should be used sparingly in flor wines. A dose of more than 80 to 100 p.p.m. shortly before seeding with flor retards film growth and a heavy dose added subsequently seems to mask flor character.

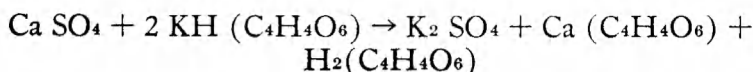
(7) *The inorganic, or mineral, constituents of the wine*

In compounding an artificial medium on which the flor film will grow vigorously it has been found that in addition to water, ethyl alcohol and suitable sources of carbon and nitrogen, the medium must also include potassium, sulphates, phosphates, magnesium, iron, and possibly, calcium. Omission of potassium, phosphates or sulphates from such a medium depressed film growth very noticeably. On the other hand omission of iron or magnesium from the medium had little effect on the initial growth of the films but affected their stability, for after a few weeks the films grown on such deficient media broke and sank in the liquid. Omission of calcium gave rather conflicting results. In some cases growth was slightly retarded, and in others it was apparently unaffected by this omission.

Up to the present none of the wines studied in this laboratory has been improved as a medium for flor growth by addition of any of the above mineral elements, and it seems likely that normal wines contain sufficient of these for the needs of the organism. There is, however, one mineral substance which has rather a special position in relation to flor sherry. This is calcium sulphate, plaster or gypsum.

According to several authorities (Castella 1909, Cruess 1948, Neihaus 1937, Marcilla 1939), the addition of calcium sulphate, in the form of 'yeso' or burnt gypsum, to the crushed grapes, is an essential feature of sherry-making in the Jerez district. This practice of plastering, as it is called, was once used extensively in Southern Europe to prevent spoilage of wines, but has fallen out of favour in many countries since the passing of legislation limiting or prohibiting its use, and since the rise in popularity of sulphurous acid and bisulphites at the beginning of the present century. At the present time, plastering does not seem to be practised extensively except in Spain, although in a few other countries plaster is probably used to a limited extent for the production of flor sherry.

When calcium sulphate is added to must, it reacts with potassium bitartrate (cream of tartar) to give potassium sulphate, insoluble calcium tartrate and free tartaric acid.



Because part of the weakly acid salt, potassium bitartrate, is converted to the more active tartaric acid, this treatment increases the acid activity or lowers the pH of the resulting wine. Moreover, because the free tartaric acid is not precipitated during fermentation as is potassium bitartrate, the titratable acidity of the resulting wine is also increased. The extent of the reaction varies from one must to another, and depends very largely on the amount of potassium bitartrate present, which in turn, is reflected in the pH value of the must—being at a maximum near pH 3.6.

If plaster or gypsum is added to the wine instead of to the unfermented must, the pH is also lowered, though to a lesser extent, while the titratable acidity is not normally increased at all. This is because much of the potassium bitartrate has already been precipitated with the rise in alcohol content during fermentataion.

Because of the widespread use of plaster in Spain, some winemakers have thought the addition of this substance to wine to be necessary for satisfactory growth of the flor. Such a belief is not supported by experimental evidence, however, for in a number of experiments in which gypsum has been added to must and to wine at rates of 2, 4 and 6 grm. per litre, there has been no significant effect on film growth other than slight effects due to changes in the pH of the wine. Thus, although plastering may in some cases be a useful method of lowering the pH of wine and thus preventing bacterial growth, it is not in itself necessary for flor growth.

Opinions on the beneficial effects of plastering on the subsequent development of flor character in the wines are varied. Reference to the prevalence of the practice in Spain has already been made, but the writer is not aware of any detailed work on the benefits of plastering in Spain.

Niehaus (1937) in South Africa recommends the addition of gypsum at the rate of four pounds per leaguer (about three pounds per hundred gallons) to the juice after pressing. Cruess (1948) reported that in California 'the wines

made from plastered musts cleared more promptly after fermentation was completed and were lighter in colour as well as more tart in flavour. The plastered wines also developed more of the flor sherry flavour and bouquet'. At Roseworthy College, South Australia, Williams (1943) found that plastering was beneficial only if the initial pH of the must was high. He considered the practice desirable only if the pH of the must was above about 3.6, in which case plastering would lower the pH sufficiently to inhibit the growth of lactic acid bacteria. Baker (1942) found that several wines made from plastered must cleared better, had a cleaner taste and developed flor character better than those made from similar musts without the addition of plaster.

Our own experience has been that the effect of plastering is chiefly a pH effect. Addition of gypsum to the wine at rates of 2 and 4 gm. per litre has not improved flor character development in laboratory experiments as judged by taste or chemical analysis unless the wine initially had a high pH value—above about 3.6—and then the effect has been very similar to that obtained by lowering the pH with tartaric acid. Addition of plaster to the must has likewise affected growth of the flor and the changes it brings about in the wine in much the same way as a corresponding pH adjustment with tartaric acid. There has been noticed, however, an important effect in a Pedro must which produced a rather coarse wine in the absence of plaster and in the presence of 4 gm. per litre of plaster produced a more delicate wine. When these wines were subsequently seeded with flor film, no obvious differences were noticed in the changes brought about in the composition of the wines by the flor, but the wine from the plastered must, being more delicate, was considered to be the better sherry.

SUMMARY OF CHAPTER VI

Both the growth of the flor film and the development of flor character have been shown to be influenced by the following factors:

1. *The strain of flor.*
2. *The amount and nature of the inoculum* with which the wine is seeded. Most rapid growth follows inoculation from a young, vigorous film grown on similar wine to the one being seeded, but other types of cultures can also be used provided they are young.
3. *The composition of the atmosphere* in the headspace above the film. Both film growth and aldehyde accumulation are inhibited by inadequate ventilation of the headspace. This effect is due to lack of oxygen rather than accumulation of carbon dioxide.
4. *Temperature.* The optimum temperature for film growth on wine is about 20° C. (68° F.). Above about 23 to 25° C. (73·4 to 77° F.) film growth often fails and films which have grown at lower temperatures, break and sink in the wine if the temperature later rises to these levels. At low temperatures film growth is slower than at 20° C., but wines on which the flor grows very vigorously commonly develop flor character more satisfactorily at about 15° C. (59° F.) than at 20° C. (68° F.).
5. *The alcoholic strength of the wine.* Although some strains of flor have been grown on wines containing 16·5 per cent. of alcohol (28·9 per cent. P.S.) growth is usually very slow, at strengths above about 15·5 per cent. alcohol (27·1 per cent. P.S.). Development of flor character is inhibited by strengths above about 15 to 15·5 per cent. alcohol (26·2 to 27·7 per cent. P.S.).
6. *Sugars and other carbon compounds* in the wine, which may serve as nutrients for the flor. There are ample carbon nutrients in a normal dry wine to satisfy the needs of the film. The presence of large amounts of fermentable sugar (about 5 grm. per litre) commonly leads to visible fermentation and film growth is delayed until the excess of sugar has been consumed and the headspace is once more adequately supplied with oxygen. This may take many weeks or even months under winery conditions. Even amounts of 2 grm. per litre commonly delay film growth and flor character development, and we have not yet found

a wine which was improved as flor sherry material by additions of sugar or of grape juice.

7. *The pH value of the wine.* Flor sherry material should have a pH between 3.1 and 3.4. Wines with lower values grow the film slowly and are usually too acid to be palatable. Higher values often lead to heavy unstable films with little development of flor character and they also increase the danger of spoilage by lactic acid bacteria.

8. *Nitrogenous nutrients.* Some wines apparently contain insufficient readily available nitrogen for normal flor growth and are improved by small additions of ammonium salts, while a few give a better response to additions of yeast extract. Film growth on most wines can be stimulated by heavy doses of either of these substances but over-stimulation of growth by such means retards rather than enhances the development of flor character.

9. *Tannin.* The presence of 0.2 grm. per litre of tannin in wine had only a slight effect on the growth or appearance of the flor, the main effect being a darkening of colour. Amounts of 0.4 grm. or more, definitely retarded growth and resulted in smooth, thin, dark-coloured films and reduction of flor character development. Important effects of high tannin content are dark colour and coarseness in the wine—two very objectionable faults in flor sherry.

10. *Sulphurous acid.* Although the flor has been grown on wines containing over 150 p.p.m. of SO_2 , growth and flor character development have been retarded by doses above 100 p.p.m.

11. *Mineral constituents of the wine and plastering.* None of the wines examined was lacking in any of the mineral substances required by the flor. The effect of 'plastering' seems to be mainly a pH effect rather than due to the addition of calcium sulphate as such. The effects of plastering can be closely duplicated by additions of tartaric acid, although in some cases plastering may be a convenient method of adjusting the pH.

CHAPTER VII

RELATIONSHIPS BETWEEN THE VARIOUS CHANGES OCCURRING IN WINE UNDER FLOR

THE changes which have been found to occur in wines under the influence of the flor have already been mentioned in Chapter V, Section 5, and in the last chapter we have discussed the effects of various factors on these changes and on the growth of the film. We are now in a position to consider the relationships of some of these changes to one another and to formulate some generalizations concerning the effects of the composition of the wine and of environmental factors on the accumulation of aldehyde and development of flor character.

The relationship between the accumulation of acetaldehyde in wine and the development of flor character has already been discussed. However, it may again be stressed that although acetaldehyde in wine is not in itself flor character, it is an important component of flor character, and the failure of wines to acquire flor character is generally associated with low aldehyde accumulation.

When the flor film grows on a wine, the changes which occur in the composition of the wine during the early stage are always of much the same kind although they differ in amount according to the vigour of film growth. During this early stage, until about the time that the film completely covers the surface of the wine, acetaldehyde accumulates, the volatile acidity falls, the redox potential falls, the fermentable sugar falls, and the glycerol content of the wine falls. These changes are accompanied by a gradual increase in the oxygen consumption of the growing film. After this initial period, however, the course of events varies considerably in different wines and under different conditions. As already pointed out, the aldehyde content of the

wine may continue to increase rapidly or slowly, it may remain almost stationary or it may fall, sometimes to rise again at a later period. Because the differences in the behaviour of wines occur chiefly during the period after complete film development, it is this period which is of particular interest to us, but in order to understand what is happening in the wine we must examine the flor film in a little more detail.

The growth of a yeast such as the sherry flor, as a film on the surface of wine or other media is an expression of aerobic or oxidative metabolism. That is to say, during this phase of its growth, free oxygen from the air plays an essential part in its life processes. We say that the film is growing under aerobic conditions and it is common to think of all the cells in the film as being adequately supplied with oxygen provided the headspace of the cask or culture vessel is well ventilated. However, it is very important to realize that in practice, all of the cells are not under aerobic conditions. Not only do clumps of cells fall from the lower side of the film and so meet anaerobic conditions in the depths of the wine, but, even in the film itself the supply of oxygen to the individual cells varies according to their position. When growth is vigorous the film is much wrinkled and folded and may be about half an inch in overall thickness. Since all the living cells are using oxygen, if it is available, and giving off carbon dioxide, it is obvious that some of the lower ones are likely to be deprived of oxygen by those above and so be placed under more or less anaerobic conditions. (See Plates 5 and 6.) Thus, in a vigorously growing film culture we have cells developing under very different conditions of oxygen supply, ranging from aerobic conditions for the upper cells to anaerobic conditions, or at least very low oxygen supply, for the lowest cells. Even though deprived of oxygen, however, these lower cells are still alive and having an influence on the composition of the wine.

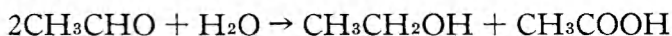
Now let us see what the flor does to wine under anaerobic conditions. This has been determined experimentally by different procedures. In one type of experiment

TABLE 15
Effect of flor film on wine under anaerobic conditions.

		Culture No.					
		1	2	3	4	5	6
At 42 days	Total aldehyde, p.p.m.	326	326	347	337	382	398
	Total aldehyde, m.mols.	7.4	7.4	7.9	7.7	8.7	9.0
	Volatile acid, gm./l.	.23	.21	.23	.24	.21	.22
	Volatile acid, m.mols.	3.8	3.6	3.9	4.0	3.6	3.7
	Closed at 42 days	Yes	No	Yes	No	Yes	No
At 52 days	Total aldehyde, p.p.m.	174	347	199	342	194	418
	Total aldehyde, m.mols.	4.0	7.9	4.5	7.8	4.4	9.5
	Volatile acid, gm./l.	.33	.16	.34	.18	.33	.16
	Volatile acid, m.mols.	5.5	2.6	5.7	3.0	5.5	2.6

previously mentioned on page 66, a suspension of flor yeast cells was shaken with wine in a closed vessel from which all the air had been removed and replaced by pure nitrogen. Under these conditions it was found that aldehyde did not accumulate, and in fact some of the aldehyde already present in the wine was destroyed. In a second type of experiment, films were grown on wine in culture bottles of the type previously described on page 32 and ventilation provided by holes through the corks which were loosely plugged with cotton. At the end of six weeks the total aldehyde and volatile acidity of all cultures were determined and half of the cultures were then deprived of oxygen by closing these bottles with air-tight corks. Ten days later the total aldehyde content and the volatile acidity of the wine in each bottle was again determined and samples were tasted. Flor character was found to be well developed in the open bottles but only slightly so in the closed bottles. Moreover, it was noticed that the films in the closed bottles had become much darker in colour than those in the open bottles. The results of the analysis of these wines are shown in Table 15.

As can be seen from these results, the cultures which continued to receive oxygen by diffusion of air through the corks, continued to increase in aldehyde content and to decrease in volatile acidity, but in those cultures which were closed at the 42nd day, the aldehyde content fell considerably and the volatile acidity increased. Furthermore, this increase in volatile acidity when expressed in millimols is equivalent to approximately half of the aldehyde which has disappeared. This supports the view that the aldehyde is destroyed under these conditions by conversion to acetic acid and ethyl alcohol according to the following equation:



In a third type of experiment the cells of the flor film were placed under anaerobic conditions by submerging them in full bottles of wine. In this experiment films were grown on 100 ml. quantities of wine in 8-oz. culture bottles for three weeks and the original wine was then carefully drawn off and fresh wine added to each bottle. Bottles 1, 2 and 3

were completely filled with wine, securely corked with waxed corks, and thoroughly shaken. Bottles 4, 5 and 6 were treated in the same way, but the aldehyde content of the wine was raised to 490 p.p.m. by addition of acetaldehyde before it was added to the culture bottles. Bottles 7, 8 and 9 each received 200 mls. of wine which was carefully run into the bottom of each bottle so as to refloat the film with a minimum of damage. These three bottles each had about 25 c.c. of headspace above the film and each was closed with a cork pierced by a 3 mm. ($\frac{1}{8}$ inch) hole loosely plugged with cotton. All bottles were then incubated at 20° C. for 28 days and then the wines were analysed for total aldehyde, volatile acidity, and glycerol. Similar analyses were carried out on the original wine.

The results of this experiment are shown in Table 16.

TABLE 16

Effect of submerged flor on the composition of wine.

Culture No.	Type of Culture	Total aldehyde p.p.m.	Vol. acidity g./l. acetic	Glycerol g./l.	Acetyl-methylcarbinol
	Original wine	75	0.30	5.7	—
1.	Submerged	59	0.36	5.6	—
2.		63	0.33	5.7	—
3.		63	0.33	5.7	—
4.	Submerged with initial aldehyde 490 p.p.m.	126	0.54	5.5	+
5.		121	0.52	5.6	+
6.		120	0.51	5.6	+
7.	Surface film	303	0.27	1.1	++
8.		292	0.22	1.0	++
9.		307	0.24	1.0	++

— = negative reaction, + = positive, ++ = strong positive reaction in qualitative test for acetyl-methylcarbinol.

Here we see that even though submerged in the wine, the flor yeast cells are still able to affect the composition of the wine. In this case the main effect disclosed was a very marked destruction of acetaldehyde in those cultures which had a high aldehyde content at the beginning of the experiment.

Other experiments in which the wines have been submitted to tasters have indicated that when the cultures are placed under anaerobic conditions, the wines lose flor character rapidly.

Now let us refer again to our vigorous flor film in which some of the cells are under aerobic conditions and others are under anaerobic conditions. Because of this uneven distribution of oxygen, two opposing reactions influence the aldehyde content of the wine. The upper, aerobic cells accumulate aldehyde, while the lower, anaerobic cells, destroy it, and the balance between these two reactions determines whether the aldehyde content of the wine rises or falls. Thus the aldehyde content of the wine, or other culture medium, does not continue to increase indefinitely, but tends to reach an equilibrium value which is determined by the conditions operating at that particular time. Actually, aldehyde does not accumulate indefinitely even under aerobic conditions. It has been found experimentally that when suspensions of flor yeast are shaken with mixtures of alcohol and aldehyde or with wine and aldehyde in air, the aldehyde content of the liquid tends to reach an equilibrium value which depends on the alcohol content and other factors. In 15 per cent. alcohol and at 15° C. the equilibrium figure was about 1,200 mgrm. per litre, while in a wine of similar strength it was 630 mgrm. per litre. These figures are both much above the levels reached in growing cultures, because in the shaking suspensions all the cells are under aerobic conditions and the aldehyde equilibrium reached represents the balance between the formation of aldehyde from alcohol and its complete oxidation to carbon dioxide and water. It must be remembered that aldehyde is not an end product but an intermediate stage in the oxidation of alcohol by the flor. The amount of aldehyde found in cultures never represents more than a small fraction (up to about 10 or 15 per cent.) of the alcohol oxidized by the flor.

Because the accumulation of aldehyde in wine under a flor film is the result of the balance between aldehyde-forming and aldehyde-destroying activities of the flor, the

amount of aldehyde present at any particular time depends not only on the length of time the film has been on the wine but also on the composition of the wine and other conditions operating at that particular time. When a high aldehyde level is reached only after a long period, this is more likely to be because conditions had previously favoured aldehyde destruction rather than because aldehyde was formed very slowly. That is to say, this period is the time taken for the composition of the wine and other conditions to become favourable for accumulation of aldehyde.

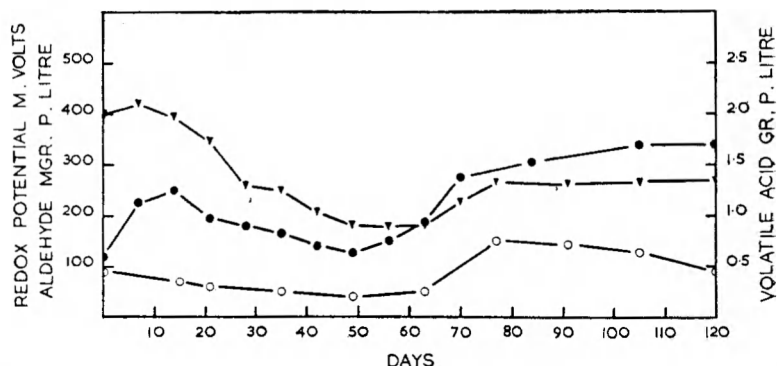


Fig. 17. Changes occurring in total aldehyde, volatile acidity and redox potential of a wine carrying a flor film for 120 days (c.f. Fig. 18).

- ——— ● total aldehyde, mgrm. per litre.
- ——— ○ volatile acid, grm. per litre.
- ▼ ——— ▼ redox potential, millivolts.

When wine is first seeded with flor yeast, there is an initial period during which the young growing film is thin and all the cells are well supplied with oxygen. Later when the cover is complete and the film becomes thick, some of the cells are more or less deprived of oxygen and, as a result, we commonly notice a marked fall in the redox potential of the wine. In other words, the flor brings about reducing conditions in the wine. It has been repeatedly observed that in wines which grow a vigorous film the rate of aldehyde accumulation during the early period, while the film is still thin, is greater than it is subsequently. In laboratory cul-

tures it has been found possible to raise the aldehyde content of some wines more rapidly by repeatedly separating the wine from the film and reinoculating it, than by allowing the wine to remain under one film for the same period. The results of such an experiment are shown in Figs. 17 and 18, which represent the changes in aldehyde content, volatile acidity and redox potential in different portions of the same wine, some of which were separated from the film and reinoculated as soon as the films were complete, while

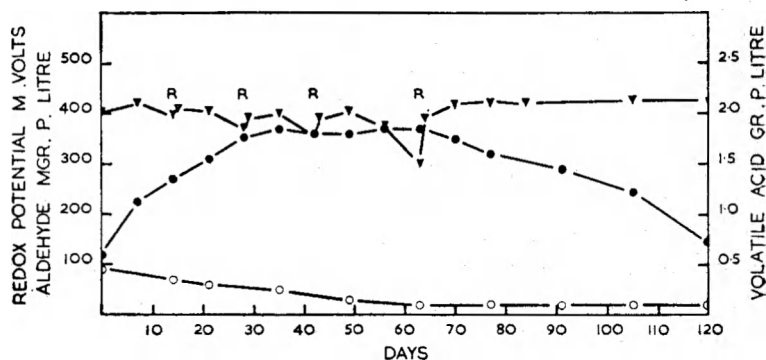


Fig. 18. Changes occurring in total aldehyde, volatile acidity and redox potential of a wine which was separated from the film and reinoculated four times. (Same wine as represented in Fig. 17.)

- —● total aldehyde, mgrm. per litre.
- —○ volatile acid, grm. per litre.
- ▼ —▼ redox potential, millivolts.
- R dates of reinoculations.

the others carried the same films throughout the experiment in the ordinary way. As can be seen from these graphs repeated inoculation resulted in a greater initial rise in aldehyde content and a longer period of high aldehyde than did continued growth of the same film. Repeated inoculation also prevented the redox potential from falling to a low level. Repeated inoculation and growth of young films gradually rendered the wine unsuitable for flor growth so that after the fifth inoculation very little growth occurred, the film sank in the wine but never covered the surface, and

the aldehyde was destroyed. The resulting wine was much over-oxidized and not at all pleasing to taste, so it is not suggested that this procedure results in high quality sherry, but the experiment illustrates the point that aldehyde accumulation depends on conditions of culture and is favoured by young, thin films in which the vast majority, if not all, the cells are well supplied with oxygen. Of course, the films on the reinoculated wines in the above experiment were much younger than on the other wines, but the effects observed are only in part due to the actual age of the films. Suspensions of cells were prepared from films of different ages and shaken with alcohol for three hours, and it was found that the cells from 66-day old films produced about 75 per cent. as much aldehyde as did those from 16-day old films.

Having shown how the aldehyde content of wine is influenced by the aerobic and anaerobic activities of the flor, we can now consider the effects of the various factors discussed in Chapter VI in relation to the balance between these activities. In doing so, we find that many of the factors which retard aldehyde accumulation or cause the aldehyde content of wine to fall, also increase the anaerobic or decrease the aerobic metabolism of the flor and so swing the balance in favour of anaerobic metabolism, and thus lower the aldehyde equilibrium.

As would be expected, restriction of oxygen supply beyond a certain point impedes aldehyde accumulation as it restricts the aerobic production of aldehyde. Wines on which the flor grows vigorously, commonly accumulate aldehyde more rapidly and regularly at 15° C. than at 20° C., although the latter temperature is nearer the optimum for film growth. This is apparently because growth and oxygen consumption are slower at the lower temperature and so the same amount of oxygen satisfies the requirements of a greater proportion of the cells than at 20° C. In a somewhat similar manner, the addition of substances such as ammonium salts and yeast extract, which stimulate film growth, commonly retards aldehyde accumulation if the films become very vigorous. By increasing the thickness of the film and thus its total

oxygen requirement, these substances force a greater proportion of the cells to live under anaerobic conditions. The presence of sugars which are, of course, readily fermented in the absence of air by the flor yeasts, also increases the anaerobic destruction of aldehyde. It is a well known fact that aldehyde added to a fermenting yeast culture is destroyed by the yeast.

The effect of high alcohol content on aldehyde accumulation is interesting. At first sight it might be expected that an increase in the alcohol content of wine would restrict growth and oxygen consumption and so favour aldehyde accumulation in much the same way as does a lowering of temperature from 20° to 15° C. In fact, however, it was found that raising the strength of a wine to 16 per cent. by volume (28 per cent. P.S.) had a depressing effect on aldehyde accumulation after the films had completely covered the wine. The explanation of this effect of alcohol seems to be that high alcohol content retards aerobic metabolism more than it retards anaerobic metabolism. In a laboratory experiment different portions of a wine were adjusted in alcohol content to 14.6, 15.3 and 16.3 per cent. by volume respectively (about 25.6, 26.8 and 28.5 per cent. P.S.); half of each batch received 2 per cent. of glucose. All were inoculated with flor and half the cultures incubated at 15° C. and the other half at 20° C. It was found that both fermentation of the residual sugar and film formation were retarded by low temperature and by high alcohol content, but the two processes were affected differently. A drop of 5° C. delayed fermentation more than did an increase of 1 per cent. in alcohol content, but film formation and alcohol consumption were retarded more by an increase of 1 per cent. alcohol than by 5° C. lower temperature.

The effect of high concentrations of carbon dioxide in the headspace, in increasing aldehyde accumulation in the laboratory experiments mentioned on page 69, Table 11, is rather a special case. Although high concentrations of carbon dioxide do inhibit film growth and aerobic metabolism of the flor it appears that they impede the aerobic oxidation of aldehyde to carbon dioxide and water more than they impede

aldehyde formation. Thus, *provided the oxygen supply is adequate*, high concentrations of carbon dioxide increase aldehyde accumulation rather than retard it. Under normal winery conditions, however, high concentrations of carbon dioxide in the headspace above the flor film only occur as a result of inadequate ventilation or the fermentation of residual sugar in the wine. In such cases the flor soon shows the effects of lack of oxygen, and aldehyde accumulation is inhibited.

Now, let us look at the sequence of events which occur when the flor film is grown on a wine under normal conditions. During the early stages the film gradually spreads over the surface of the wine, but is still thin enough for nearly all the cells to be well supplied with oxygen. During this period the aldehyde content of the wine rises steadily, the volatile acidity falls and the redox potential falls slightly. However, usually before the aldehyde content has reached a very high level, conditions have changed, for the film has become thicker and wrinkled and its oxygen consumption and requirements have increased to such an extent that many of the cells in the lower portions of the film cannot get enough oxygen for aerobic metabolism. Thus, depending on conditions such as thickness of the film, temperature and composition of the wine, a greater or less proportion of the cells of the film are now forced to carry out anaerobic or aldehyde destroying reactions. Consequently we commonly find that aldehyde accumulation becomes slower at this time and in some cases the aldehyde content of the wine may remain almost stationary for a period or even decrease. Further evidence of the increased anaerobic activity of the flor is seen in the marked fall in redox potential and the reduction in colour of the wine at this time. The length of this second stage varies considerably according to the composition of the wine and other conditions, but is always longer in casks or other large containers than in shallow laboratory cultures of the same wine. Eventually, however, in many wines, the second stage is succeeded by a third stage. Because of the activity of the flor and probably by the exhaustion of some essential nutrients, the wine becomes a less

favourable medium for growth, oxygen consumption decreases and allows more effective penetration of oxygen to the lower cells of the film. For this and perhaps other reasons anaerobic metabolism of the flor declines and the redox potential rises again. This is commonly accompanied by a second rise in aldehyde content which is particularly marked in laboratory cultures but has also been observed in some wines in casks. In small laboratory cultures, this second rise in aldehyde content is commonly accompanied by a rise in volatile acidity which is particularly marked in cultures carrying a very heavy film growth. As previously mentioned (page 52) it seems likely that in such cases the volatile acid is derived at least in part from the breakdown of the yeast cells themselves and no appreciable rise of this kind has yet been detected under industrial conditions.

Ultimately the second rise in aldehyde ceases and the third stage gives place to a final stage when the wine has become so unsuitable for flor growth as a result of the prolonged activity of the organism, that the film becomes wet and sinks in the wine, in some cases after becoming dark in colour. By this time both the aldehyde and volatile acidity have usually fallen considerably, and on tasting the wine it is found to have become flat and lacking in flor character.

A glance at the various graphs will show that the stages discussed above are not equally clear cut under all conditions. The initial stage of aldehyde increase lasts until the wine is covered by a heavy film so that its duration is related to the surface area of the wine, whereas the amount by which the aldehyde content of the wine rises is, of course, related to the volume of the wine. Thus, we find that the initial rise is greater in shallow than in deep quantities of wine, if other things are equal. Even during the early stage the balance between anaerobic destruction and aerobic production of aldehyde may be affected so as to inhibit aldehyde accumulation if the wine contains appreciable amounts of fermentable sugar (Fig. 15).

The influence of various factors on aldehyde accumulation during the second stage have already been discussed in some detail. It has been shown that, depending on conditions

affecting the balance between aerobic and anaerobic processes, this stage may be practically a continuation of stage one, though usually with a rather slower accumulation of aldehyde, or on the other hand it may be characterized by a stationary, or even a falling, aldehyde content.

The third stage or second rise in aldehyde content is only obvious when aldehyde accumulation has already been retarded during the second stage. This second rise in aldehyde always occurs later in deep cultures than in shallow ones if other things are equal. In the laboratory it has been found that there was not a well-marked second rise in aldehyde accompanying the rise in redox potential if the alcohol content of the wine was high (16 per cent. by volume or 28 per cent. P.S.) or if the films were very unstable and sank in the wine early. In only a few instances has a well marked second rise in aldehyde been observed under winery conditions, but it is possible that this is because the time taken for casks of some wines to reach a condition which favours a second rise in aldehyde is longer than the wines are normally 'left on flor'. In other words, some wines which might exhibit such a second rise are fortified before this can occur.

The final stage of film-drop and loss of aldehyde and flor character is not often seen under winery conditions for the obvious reason that the winemaker does not intentionally allow his wine to carry the film until this stage is reached.

The changes occurring in wine under flor have been discussed in the above stages to explain the way in which they are influenced by various factors, but in actual fact the practical winemaker does not wish to see clear-cut stages of development. His objective is to impart to his wine the maximum of flor character as quickly as possible, and so he wishes the flor character to increase steadily and continuously without any stage of loss of aldehyde and flor character intervening. Our experimental results indicate that this desirable state of affairs occurs most readily when film development is moderately vigorous, resulting in light-grey or fawn-coloured lightly wrinkled films of moderate thickness rather than with the very heavy, white to pale cream-coloured films seen on some wines.

In the foregoing discussion, attention has been focused on the rôle of anaerobic processes in destroying acetaldehyde in the wine, but it should not be inferred that all the anaerobic activities of the flor are undesirable. Although the anaerobic reactions brought about by the flor are at present very imperfectly understood it is highly probable that at least some of them contribute to the quality of flor sherry. The reduction in the colour and specific gravity of wine which is so desirable for the pale dry sherries, is closely related to the vigour of film development and is brought about at least in part by anaerobic processes. The parts played by aerobic and anaerobic processes in the formation of esters, acetals and other constituents which may contribute to flor character are not known, but it is highly probable that anaerobic processes play some part. Ribéreau-Gayon (1947) has shown that the development of bouquet in bottled dry wines is essentially due to reduction processes and it is likely that similar processes also play some part in the development of flor character in wines carrying the flor film. It has been suggested by Cruess (1948) that the autolysis or break down of yeast cells in the depth of the wine also contributes to the flavour of flor sherry.

Thus we reach the conclusion that the winemaker should not seek to prevent the anaerobic activity of the flor entirely, even if he were able to do so. He should rather endeavour to obtain the right state of balance between aerobic and anaerobic processes so that aldehyde accumulation and the development of flor character as a whole is a continuous process. Control of this balance can be achieved in large measure by control of the various factors which have already been discussed and which may be broadly grouped as choice of a suitable strain of flor, choice of a suitable base wine, provision of suitable conditions particularly as regards air supply and temperature, and separation of the wine from the flor when flor character development is at a maximum. Recommendations for the practical use of the flor under winery conditions will be discussed in the next chapter.

THE USE OF THE FLOR FILM IN THE WINERY

1. CHOICE OF THE BASE WINE

The first and one of the most important considerations in making flor sherry, is the selection of a suitable base wine. High quality sherry can only be made from high quality base material. It is not enough that the wine should grow a flor film or even that it should acquire 'flor character'. The product must be palatable. This may seem so obvious as to be scarcely worthy of mention, and yet one occasionally finds wines having flor character but which are rendered unpalatable by some defects such as coarseness or excessive acidity. The base wine should be clean, light and delicate, without any pronounced varietal flavour such as we find in Muscats or Reislings for example. In Spain, the Palomino or Listan is the choice variety for fino sherry, although the Pedro Ximenes is also used to a limited extent. Unfortunately, the Palomino, also sometimes known as Sweetwater, is not grown extensively in this country as yet, and there is little doubt that some of the vines known as Palomino here are incorrectly labelled. A few small trials conducted in both wineries and the laboratory have indicated that further plantings of Palomino might be an important factor in raising the quality of our sherry. Of the varieties which are widely grown in Australia, the Pedro seems to be the most generally suitable for sherry, though here again, more than one variety masquerades under the same name. Other varieties sometimes used include Albillio, White Hermitage (Ugni Blanc), Doradillo and Reisling, but these are less satisfactory. Reisling, because of its own varietal bouquet takes the flor character less well than varieties of more 'neutral' flavour, and the other three varieties mentioned are rather prone to coarseness, particularly in some of the warmer districts. There is some evidence that the wines from such varieties

are improved as regards lightness and delicacy of flavour if the juice is treated with pectin-splitting enzymes, allowed to settle, and is then drawn off the sediment before fermenting. Williams (1943) reports success with this treatment at Roseworthy Agricultural College.

The alcohol content of the wine must be high enough to protect it against invasion by acetic acid bacteria, but laboratory and winery experience have shown that there is no advantage, but rather a disadvantage, in raising the strength much above this safety level. It is recommended that the alcohol content of the wine be adjusted by blending or fortifying to between 14.5 and 15.2 per cent. by volume (25.5 to 26.6 per cent. P.S.), but if spirit is added it is most important that this should be very clean and neutral.

The base wine should be dry, containing not more than about 2 gm. per litre, and preferably less than 1.5 gm. per litre of reducing sugar. Larger amounts usually delay the development of flor character in the wine and also increase the risk of bacterial spoilage. It is important that the tannin content of the wine should be low, and to this end only the free-run juice and light pressings should be used for making wine intended for sherry, since heavy pressings and a high tannin content commonly impart coarseness to the wine. The pH of the wine should be between 3.1 and 3.4, for it has been found that this range is sufficiently acid to protect the wine against lactic acid spoilage and yet permits satisfactory growth of the film and development of flor character. More acid wines are likely to be unpleasantly tart, but it should be remembered that sourness, or acidity as registered by the human palate, is not the same thing as pH value. Although the effect of acidity on the growth of micro-organisms and on the activity of enzymes is closely related to pH value, sourness on the other hand, depends also on the amount of acid present or the titratable acidity but cannot be accurately measured on either of these scales. The sensation of sourness given by a particular amount of acid varies from one wine to another, and is influenced by several factors, including the amounts of sugar and alcohol in the wine. As a working basis, it is recommended that the base wine should have a pH between 3.1 and 3.4, and a

titratable acidity between 4.5 and 6.0 grm. per litre as tartaric acid. Each wine must be considered individually, however, and tasted, for some containing even larger amounts of acid develop into palatable sherries, while a few containing less than 5.5 grm. per litre are too sour.

The SO₂ content of sherry material should not be high. Cruess (1948) recommends 150 p.p.m. as a means of preventing bacterial spoilage, but we have found the flor character to develop more regularly when the SO₂ is kept below 100 p.p.m. even although the film can be grown in the presence of over 150 p.p.m.

It has been our experience that bacterial spoilage can be controlled without recourse to high doses of SO₂, provided that the alcohol content is kept above 14.6 per cent. by volume (25.5 per cent. P.S.); the pH is kept between 3.1 and 3.4 and the initial sugar content of the wine is below 2 grm. per litre.

When the wine has been selected and after any necessary adjustments to its composition have been made, it is clarified if necessary, either by fining or filtration. Some makers pass the wine through a germ-proof filter at this stage, and this practice, although often not essential, is to be commended as an additional safeguard against spoilage. It should be remembered, however, that such a filtration is of little value unless the wine is run from the filter through sterilized hoses or pipes, into sterilized casks.

Although the majority of wines which answer to the specification already given, grow the flor film satisfactorily, there are a few wines on which growth is very slow. Such wines can be detected by means of laboratory trials in which the wine, in cotton-stoppered flasks, bottles or jars, is seeded with flor and incubated at 20° C. Two to three weeks of incubation should provide an indication of just how the film is likely to grow on the wine, and will indicate the need for modifications such as additions of ammonium phosphate. Such laboratory trials can give a very valuable lead as to the suitability of the wine, and thus save valuable time in establishing the film in the winery. If film growth is poor in the laboratory, that is to say, if the film remains very

thin and smooth and covers the surface of the wine very slowly, the alcoholic strength, SO_2 content and pH value of the wine and the temperature of incubation should be checked. If it is obvious that none of these factors is to blame, it will usually be found that growth will be improved by the addition of a small amount of diammonium phosphate (about 2 to 4 ounces per 100 gallons). If there is no response to a dose of this size it is generally not advisable to use larger amounts, and it is important to remember that over-stimulation of growth with ammonium salts is to be avoided. After all, the objective is to produce flor sherry rather than a heavy crop of flor film and, as previously pointed out, better results are usually obtained with a moderately vigorous film than with very heavy growth. A few wines which do not respond to additions of ammonium phosphate give improved growth after the addition of yeast extract, which may be conveniently supplied in the form of 'marmite' or 'vegemite'. Although both of these products have a very pronounced flavour they do not adversely affect the flavour of the finished wine when they are used in small amounts (2 to 4 ounces per 100 gallons). Again, it is important to remember that over-stimulation of film growth with heavy doses is to be avoided.

2. CONTAINERS

The production of acetaldehyde which has been shown to be one of the most important features of the development of flor character, occurs at the surface of the wine. On the other hand, the flor yeast cells which have fallen from the film and accumulated on the bottom of the container, are able to bring about other changes, including the destruction of acetaldehyde. Because of this, both the rate and the amount of flor character development are influenced by the ratio of the area of the flor film to the volume of the wine. When this ratio is very high, aldehyde accumulation is rapid, but the wine acquires a pronounced flavour of yeast. On the other hand, when the area of film per gallon of wine is very low, the development of flor character is unduly delayed. For this reason the type of container used and the

amount of wine placed in it have an important bearing on the development of flor character. According to Marcilla (loc. cit.) the typical containers used in the Jerez district of Spain are the oak 'botas jerezanas' or sherry butts with a capacity of about '30 arrobas' or from 480 to 500 litres (about 106 to 110 imperial gallons). These casks are filled about five-sixths full on the average, so as to leave about 18 gallons ullage space above the wine

In this country casks of various sizes are used for making sherry, but by far the most common are hogsheads and, to a lesser extent, puncheons. The capacities of these are usually about 60 to 65 gallons and 105 to 115 gallons respectively, so that our puncheons resemble the Spanish 'botas' quite closely. It is the usual practice when running flor sherry material into such casks, to leave ullage spaces of about 10 gallons in the hogsheads and 20 gallons in the puncheons. Calculations based on measurements of typical puncheons and hogsheads have shown that 95 gallons of wine in a 115 gallon puncheon has surface area for film growth of about 7 square feet or about 10·6 square inches per gallon of wine. The corresponding figures for 55 gallons of wine in a hogshead were about 4·7 square feet of surface area or about 12·3 square inches per gallon of wine. These figures would vary slightly from one cask to another because of slight differences in size and shape of the casks, but they vary considerably with differences in the amount of wine in the same cask. Because of the shape of a normal cask, an increase of a few gallons in the amount of wine placed in it at this level reduces the surface area available for film growth appreciably. For example, in a hogshead of 64·2 gallon capacity, 60 gallons of wine had 6·8 square inches of free surface per gallon of wine, while 56 gallons had 11·5 square inches per gallon and 51 gallons had 15·1 square inches per gallon. It is for this reason that the amount of wine placed in a cask for flor growth is important. There is no evidence that the volume of the ullage space is in itself important. Indeed, since tests have shown that 1 square foot of vigorous film is capable of using the oxygen in 0·5 to 1 cubic foot of air in 24 hours, it is obvious that the initial

oxygen supply in the ullage space will not meet the requirements of the film for very long. The film must depend on air which enters the cask during flor growth and thus it is freedom of ventilation rather than volume of ullage space which governs the oxygen supply of the film.

Current methods of providing ventilation of casks vary considerably in different wineries, the most common being the use of a loose shive and hessian closure at the bung-hole, a hardwood bung pierced by a hole half an inch in diameter and plugged with cotton-wool, or alternatively, the wooden bung is removed and the bung-hole closed with a plug of cotton-wool. The amount of ventilation allowed by these different closures varies considerably, but in winery experiments we have found the third type, that is the cotton-wool plug, to give most rapid film growth and flor character development in both puncheons and hogsheads.

Within recent years both in California and Australia flor sherry has been made in cement tanks as well as in wooden casks. Martini (1950) reports the use of tanks containing a depth of 18 inches of wine. In this country cement tanks of various sizes have been used from time to time, usually containing a depth of between 18 and 24 inches of wine. Of course, the area of film per gallon of wine is decreased when the depth of the wine is increased. In a tank with perpendicular walls the surface area per gallon of wine with various depths of wine is as follows:

Depth of wine (inches)	Square inches of surface per imperial gallon
10	27.6
15	18.5
18	15.4
20	13.8
22	12.6
24	11.5

Thus a depth of 23 inches in a tank provides a surface : volume ratio comparable with that obtained with 55 gallons in a hogshead, while a depth of 26 inches in the tank is comparable in this respect with 95 gallons in a puncheon.

Our experience indicates that when tanks are used the depth of wine should generally be between about 18 and 24 inches. Greater depths delay the development of flor character while shallower layers of wine, although often accumulating aldehyde rapidly, are likely to acquire a very pronounced flavour of yeast if film growth is heavy.

3. CHOICE OF THE STRAIN OF FLOR

It is claimed by some winemakers that different strains of flor are suited for production of flor sherry from different base wines. Although this may be so, we have at present no experimental evidence to support this claim. In all of the strain tests carried out, both in the laboratory and in several wineries, it has always been the same strains which have given the best results although the differences between strains have been much greater in some wines than in others. A number of strains of flor are maintained in our collection at the Waite Agricultural Research Institute and cultures of these are made available to winemakers. Unless a request is made for a particular strain, it is our policy to supply the strain which has given the best results in our trials.

4. ESTABLISHING THE FLOR FILM IN THE WINERY

Cultures of flor are usually supplied from this laboratory as young growth on diluted grape juice agar in tubes or bottles which have been inoculated from film growth on wine. When received at the winery such a culture may be used to inoculate a few hundred ml. of pasteurized or filter-sterilized wine in a bottle or flask provided that the culture is used within a few days of being received. This inoculation is carried out by transferring some of the growth from the surface of the agar to the surface of the wine by means of a sterilized wire loop. The flor is gently rubbed on the glass wall of the flask or bottle just at, and immediately above the surface of the wine. The wine is then kept at a temperature as near 20° C. (68° F.) as possible and not above 22° C. (71.6° F.), till the film has grown—usually two to three weeks. If the agar culture is not to be used imme-

diately on arrival, it will keep quite well for several weeks if left unopened and stored in a cool place, preferably in a refrigerator. In this case, however, it is advisable to inoculate sterilized grape juice rather than wine. Some of the growth is transferred to about 100 ml. of grape juice of 13° to 14° Baumé, previously pasteurized or boiled, in a small bottle or flask which is closed with a wad of sterile cotton-wool. The grape juice is kept at about 20° C. (68° F.) until it has fermented and grown a film, a period which usually occupies four to six weeks. This film is then used to inoculate some of the wine to be used as flor sherry material in a large flask, a flagon or a cask. Such inoculation can be carried out either by transferring portions of the film with a sterilized wire loop, glass rod, or a piece of stainless steel gauze of fine mesh, and floating the film on the surface of the wine, or by thoroughly shaking up the film in the small bottle so as to form a suspension of flor yeast cells and adding this suspension to the wine. When the latter procedure is adopted, the aim should be to leave a large proportion of the cells floating on the surface of the inoculated wine and to have them well distributed over the surface of the wine. This may be achieved by pouring the suspension slowly down a sloping glass rod, the end of which dips into the wine, and which is moved about to distribute the inoculum during the operation, or alternatively, the suspension may be sprayed over the surface of the wine with a small atomizer. In some cases suspensions of flor film in wine are supplied by this laboratory, and these can be used as indicated above, but should be used as soon as they are received.

It is a mistake to try and 'stretch' the inoculum by inoculating a number of casks or other large containers from a small quantity of film growth. A very light seeding commonly leads to slow film growth. This not only wastes time but increases the risk of bacterial spoilage, for it is during this period, when the wine is left exposed to the air and is not yet covered by flor, that danger from invasion by acetic acid bacteria is at its highest. To quote a picturesque phrase from Castella (1926), 'The sherry during this stage is working

out its destiny on the borderland of the vinegar country.' The film growth on wine in, say, a four or six-ounce bottle as indicated above, can be used to seed one hogshead but should not be spread over a greater area of wine surface. This should be regarded as a minimum seeding and heavier seedings will lead to more rapid cover. It is obvious, therefore, that the winemaker should commence preparing cultures for seeding his stock of sherry material several weeks before he wishes to carry out this operation. As indicated above, these cultures may be prepared in a large number of small flasks or bottles, in a few large flasks or flagons or in casks. For best results, however, the cultures should be used while still young, preferably during the first three weeks after complete films have been formed, as older cultures commonly lead to slower film growth on the seeded wine. This applies particularly to cultures in small bottles or flasks.

5. TEMPERATURE

The importance of temperature in relation to the growth of the film and the development of flor character has been discussed in Chapter VI. Records of measurements of wine temperature in typical Australian sherry cellars show seasonal variations from 10.5°C . (51°F .) in July and August, up to 24°C . (75°F .) in February and March, and there is little doubt that this latter figure is exceeded in some cellars. As previously pointed out, the optimum temperature for flor growth on wine is about 20°C . (68°F .), and so it is not surprising that in some wineries and in some seasons the flor films become dark and sink in the wine in the latter part of the summer as a result of high temperatures. This trouble is less common now than it was several years ago because of a better understanding of the effects of temperature and more rational selection of the parts of the winery to be used for flor sherry. However, it is often not appreciated that temperatures which favour a very rapid and prolific growth of the film, do not always lead to maximum development of flor character. The laboratory experiments previously discussed have shown that in many wines the development of flor character was greater and more rapid

at 15° C. (59° F.) than at 20° C. (68° F.), although film growth was always more vigorous at the latter temperature. The wines which behaved in this way were in general those on which the flor grew vigorously. Wines which supported only a medium or weak film growth usually developed flor character more rapidly at 20° C. (68° F.) than at 15° C. (59° F.), although those incubated at the latter temperature eventually had as much character as those at 20° C. While it has not been practicable to carry out experiments in wineries with temperatures adjusted and controlled at various levels, the results of many observations of the behaviour of different wines carrying flor in various wineries are in agreement with the foregoing experimental results. It appears then, that at about 15° C., (59° F.) all wines will develop their maximum flor character, although the process is unnecessarily slow in some. On the other hand, temperatures in the vicinity of 20° C. (68° F.) will hasten the development of flor character in some wines, but will greatly delay or even prevent satisfactory development in others.

When we consider the effects of temperatures below about 15° C. (59° F.), we find, as would be expected, that they delay film growth appreciably. Nevertheless, it has been noticed that some wines have developed considerable flor character even at 12° C. (53.6° F.) if the film had already been grown at higher temperatures.

In view of the importance of temperature it is necessary that the winemaker exercise some form of control over it. The ideal method of doing this is by constructing an insulated building and installing heating and cooling devices, but failing this, the effects of high summer temperatures can be avoided to a large extent by the use of an underground cellar, or a well-insulated building. When such premises are not available the winemaker can make the best use of the available accommodation by seeding his wine in the autumn. Where temperature fluctuations are small, the wine will usually be seeded as soon as it can be prepared, generally in the early spring of the year it was made. However, if the temperature fluctuates widely, it is desirable to seed the

wine in the autumn, when the temperature of the wine is between 18° and 20° C. (64° and 68° F.). This will enable the film to cover the wine before the low temperatures of winter check its growth and will allow the maximum period for the development of flor character before the high temperatures of summer intervene. Unfortunately, there are practical difficulties here, for it is often not possible to prepare new wine for flor before August or September at the earliest. Wine seeded at this time, in premises where wide fluctuations of temperature occur, will have only a few months in which to develop flor character before the summer heat interferes with the process. For this reason it is often advisable to hold the wine over till the next autumn before seeding. On several occasions when the progress of flor character development has been followed in casks of wine over periods of one or more years, it has been noticed that the main development was between May and October, and that there was a definite loss of character during the period January to March, even though the films remained healthy in appearance.

The undesirable effects of high temperatures are particularly noticeable when the flor is grown in tanks instead of casks. Although the tanks are usually protected with some type of cover the free ventilation commonly allowed ensures very vigorous film growth. As a result, flor character development is readily checked by high temperatures and, moreover, alcohol losses become an important economic factor.

6. LENGTH OF TIME TO LEAVE THE WINE 'ON FLOR'

From the discussion of the effects of various factors on the development of flor character, it will be evident that the time taken for maximum development varies considerably with the wine and conditions such as temperature and ventilation. Experimental results do not support the claim sometimes made, that 'the longer the wine is "on flor" the better'. Several experiments carried out in wineries have shown a definite loss of flor character from wines after a maximum had been reached.

It is not possible to indicate any exact period for maximum development of flor character, and the winemaker must treat each wine and, in fact, each cask, individually. Regular withdrawal of samples for tasting and for the determination of total aldehyde and volatile acidity provide a very useful method of following the progress of the wine. The former analysis will indicate the course of flor character development, while a rise in volatile acidity will give warning of spoilage. As long as the wine remains sound, the film appears healthy and the total aldehyde content is rising, the wine may safely be left 'on flor'. A slight rise (0.1 to 0.5 grm. per litre) in volatile acidity need not cause alarm, but indicates that the wine should be carefully watched and be drawn off and fortified at once if the rise continues. It is not uncommon to find that the aldehyde content of a wine fluctuates slightly and then continues to rise again, but if the aldehyde content starts to fall while the film is thick and particularly if this occurs when the temperature of the wine is rising in the early summer, then it is likely that a considerable fall in aldehyde and loss of flor character will occur. Since this loss can occur very rapidly (in a few weeks) particularly in tanks, doubtful wines should be examined at frequent intervals. In wineries where the sherry is subject to marked seasonal fluctuations in temperature, the chief losses in flor character occur during the summer months so that the most appropriate time to draw off and fortify these wines is usually at the beginning of summer. Using hogsheads with cotton-wool bungs and wines of the type indicated in the first section of this chapter, we have found maximum aldehyde accumulation and flor character development after from six to twelve months. In the few experiments carried out in puncheons, periods of nine to eighteen months have been required for maximum development, while in tanks containing a depth of between 18 and 20 inches of wine, the process has been more rapid and the maxima have been reached in from three to six months.

The foregoing discussion is concerned with the practice of drawing off the whole of the contents of a cask or tank at one time, which is the usual procedure in this country. In

Spain, however, use is made of the Solera system of maturation, which has been fully described by Castella (1909). Under this system only a portion of the wine is drawn off from a cask at any one time and is replaced by a like amount of young wine. The amount and frequency of withdrawals are so adjusted as to yield a regular supply of flor sherry from each cask and the film remains unbroken in the cask. Up till the present we have not had any great experience with this system in Australia, but some modified form of it would be worthy of investigation.

7. SUBSEQUENT HANDLING

When it is decided that the sherry should be separated from the flor film it should be drawn off and fortified with very clean, neutral, high-proof spirit, or blended so as to raise the alcohol content to about 32 per cent. proof spirit. It is important that the wine be free of suspended yeast cells at this stage, for when placed under anaerobic conditions the flor cells destroy flor character. Although absolute brilliance of condition need not be sought at this stage any considerable amount of flor in suspension is a disadvantage and should be removed by filtration. Usually flor sherry is a wine suitable for blending rather than direct consumption. When drawn off the flor it is too dry in flavour for most people and, if flor character is very marked, the wine is always improved by judicious blending. Wines matured under flor may contain between 300 and 500 parts per million of acetaldehyde which, while making them unpalatable in themselves, renders them very valuable for blending. Considerable care is needed in selecting wines for blending with flor sherry. Obviously, these must be selected with due regard to price and quality of the finished wine but it is important that the flor character is not too greatly diluted in the blend and that it is not masked by other pronounced flavours. The wines used for blending should be mature and so chosen as to give the desired alcoholic strength, acidity and sugar content to the finished wine.

Once the wine has been separated from the flor it should be stored in full and tightly bunged casks until ready for

bottling, for at this stage it is sensitive to oxidation and may be spoiled in a few weeks if left in ullaged casks. Following blending and fortification the wine should be matured in well-seasoned casks for a further period of one to several years and then fined or filtered prior to bottling.

8. SOME COMMON DIFFICULTIES IN PRODUCING FLOR SHERRY

The influence of various factors on the flor yeasts has already been discussed in some detail, but for the convenience of winemakers the more important causes of failure of film growth and of flor character development will be briefly reviewed here.

Failure of film growth is most often due to one or more of the following:

(a) Lack of oxygen because of inadequate ventilation of the container, or because of the production of carbon dioxide during the fermentation of residual sugar in the wine.

(b) High temperatures. The film seldom grows vigorously when the temperature of the wine rises much above 22° C. (about 72° F.).

(c) High alcohol content. Although film growth sometimes occurs on wines containing over 16 per cent. of alcohol (28 per cent. P.S.) it is definitely retarded above about 15 per cent. alcohol.

(d) Inferior strain of flor.

(e) High SO₂ content.

(f) Excess of fermentable sugar. The presence of more than about 2 grm. per litre of sugar often results in a slow fermentation which keeps the ullage space above the wine filled with carbon dioxide. Under these conditions film growth may be delayed for months until the sugar has disappeared and sufficient of the carbon dioxide has diffused out of the cask to allow the oxygen content of the headspace gas to rise.

(g) Lack of nitrogen. There are some wines which appear to contain insufficient available nitrogen for normal flor growth and which are improved by small additions of ammonium phosphate.

Failure of the wine to develop flor character may, of course, be due to poor film growth, in which case the cause is likely to be one of the factors listed above. On the other hand, as pointed out in the preface, wines may grow the flor film well and still fail to develop flor character. This trouble is often due to excessive anaerobic metabolism of the flor and is frequently, though not always, associated with very vigorous film growth. Common causes of lack of flor character development are:

(a) High temperature. Many wines develop flor character more regularly at about 15° C. (59° F.) than near the optimum for growth which is about 20° C. (68° F.).

(b) High alcohol content. Even though the film may grow, the development of flor character is inhibited by high concentrations of alcohol.

(c) Excess of fermentable sugar.

(d) High nitrogen content. Over-stimulation of film growth by addition of too much ammonium phosphate to the wine retards the development of flor character appreciably.

(e) High pH value.

(f) Inferior strain of flor.

Another difficulty sometimes encountered by winemakers is the spoilage of the wine by bacteria. The two most common types are due to infection by acetic acid bacteria and lactic acid bacteria respectively. Actually, the conditions required for growth of acetic acid bacteria are quite similar to those required by the flor, except for the important difference in the tolerance of the two organisms for alcohol. The winemaker's defence against spoilage by these bacteria consists of ensuring that the wines are sound and are run into sound (preferably sterilized) casks prior to seeding with flor, then obtaining a rapid cover of the surface with flor film and seeing that the alcohol content of the wine never falls below 14.6 per cent. by volume (25.5 per cent. P.S.). The control of spoilage by lactic acid bacteria has been discussed by the present writer in another publication (1943). Trouble due to this type of spoilage in flor sherry can be avoided by general winery hygiene, by seeding only sound

wines in sound containers and by making sure that residual sugar content of the wine is below 2 gm. per litre and the pH value below 3.4. Up till the present, it has been our experience that bacterial spoilage of both the above types can be avoided in flor sherries without recourse to massive doses of SO_2 provided the foregoing precautions are observed.

In connection with bacterial spoilage, mention should be made of the widely advocated use of the sherry flor for reclaiming spoiled wines. Cruess and his associates (1937) have shown the ability of the flor to lower the volatile acidity of 'pricked wines' and this has been confirmed by many other workers. Cruess (1937) and also Bobadilla (1943) recommended that, provided acetification was first arrested by addition of SO_2 and raising the alcohol content, then the 'pricked' wine could be reclaimed by means of the flor film. Russian workers (Saenko 1945) claim to have also reclaimed wines spoiled by lactic acid bacteria. Our own results confirm those of the above investigators so far as the ability of the flor yeasts to destroy lactic and acetic acid is concerned, but attempts to reclaim spoiled wines are often disappointing. It has been shown by Peynaud (1936) that the characteristic flavour and aroma of 'pricked' wine is due largely to ethyl acetate rather than to acetic acid. It has been our experience that although the flor yeast lowers the volatile acidity of 'pricked' wines quite effectively, it fails to destroy the ethyl acetate, so that badly spoiled wines remain unsound in flavour and aroma after flor treatment even though they have quite a low volatile acidity.

Although the main interest in the flor yeast is centred round its use for making sherry, it must be remembered that the same organism is capable of growing on other unfortified dry wines, both red and white, if they are left exposed to the air. When it occurs on such wines, particularly on light delicate table wines, the flor is rightly classed as a spoilage organism and should be treated as such. In Australia and other countries where several other types of wine besides sherry are made or handled at the same winery, the winemaker needs to take precautions to avoid contamination of other wines with flor. Preferably, separate premises

and equipment should be reserved for flor sherry, but certainly, any containers, hoses or other equipment that have been used for flor should be thoroughly cleaned and disinfected before being used for other wines. Finally, casks or vats containing dry wines should be regularly topped up and tightly closed once fermentation is complete.

CONCLUSION

The investigations discussed in the previous pages have been concerned with the nature of the flor yeasts and the changes which they bring about in wines under various conditions. As a result of these investigations certain recommendations have been made concerning the production of flor sherry. Such recommendations are, for the most part, formulated as general principles rather than specific directions and should be interpreted in the light of local conditions by the winemaker.

The factors which influence the quality of sherry may be grouped as:

- quality of the base wine,
- the strain of flor,
- the conditions under which the flor is grown,
- the subsequent handling of the wine.

Only by attention to all of these can quality flor sherry be made, but the importance of the first is here once more emphasized since it is probably the factor most often lost sight of. High quality flor sherry can only be made from high quality base wine. In this connection, the variety of grape is of great importance and has only been touched on in these investigations. The suitability of different varieties in different districts may well prove to be a profitable field for further study.

It has been found that chemical analyses provide a valuable guide both in selecting the base wine and in assessing the quality of the product, but in the present state of our knowledge, such analyses are only a guide and not an exact measure. The final assessment of quality in sherry as in other wines is, of course, by the human nose and palate.

LATER RESEARCH

THE more important developments since the publication of the first edition are summarised below, with a supplementary bibliography following the main bibliography.

A considerable amount of work on the microbiological classification of the flor yeast has continued. This work has recently been reviewed by Amerine and Kunkee (1968), who cover what has been accomplished in considerable detail, but are forced to conclude that the correct classification of the film-forming yeasts used in sherry production in Spain and elsewhere is still an unsolved problem. A history of similar work in Russia is given by Saenko (1964).

Studies of the nutritional requirements for growth of flor yeasts have extended the findings reported in this book. Saenko (1951) reported that there is an optimal level of nitrogen in the wine of about 120 mg per litre for the growth of the flor yeast. Frieberg and Cruess (1955) studied many growth accelerating and inhibiting substances and came to the conclusion that urea or ammonium phosphate could be beneficial as nitrogen sources under some conditions, but that calcium sulphate had no effect. In contrast, a review by Gonzalo (1954) on the practice of "plastering" concluded that juice treated with calcium sulphate showed more rapid fermentation. Later work (Samvelyan and Agabalyants, 1957; Ivlev 1958) agreed with Fornachon's conclusions that the supply of oxygen was the most important nutritional factor influencing the quality of sherry. Castor and Archer (1957) reported that pantothenate, one of the B vitamins, was essential for growth on a synthetic medium, but that they could not produce any synthetic medium as nutritionally adequate as wine. Saenko (1959) examined the influence of temperature on flor growth and biochemical activity. His findings agree closely with the recommendations given by Fornachon. A recent Russian report (Gadzaiev 1970) claims that the activity of the film yeast is increased by high levels

of boron and suggests vineyard treatment with boric acid.

There have been several reports from Spain and Italy of the use of flor yeasts to age dry red wines more rapidly, with claims of producing high quality wines. The flor yeast is allowed to grow for a limited period of only a few weeks and reduces volatile acidity, but causes loss of colour and tannin (Castelli 1960). However detailed data are lacking, and in view of the possibility of spoilage, the proposal seems questionable. Russian workers (Martakov *et al* 1963) report that wines similar to sherry may be made using a dry red base wine.

The newer methods of cultivation of yeasts in sherry production, submerged and continuous culture, arose from the observations of Fornachon (1953) that flor yeasts shaken with wine rapidly produce acetaldehyde. Crowther and Truscott (1955, 1957) developed means for rapid production of flor-type sherries by agitation; they reported that, using base wines made from American hybrid grape varieties, the final product was similar to Spanish flor sherry. Similar results were obtained by Amerine (1958) and Ough and Amerine (1958, 1960) by aeration with periodical stirring to keep the yeast culture submerged. Levels of acetaldehyde as high as 500 mg per litre could readily be obtained, but the product was not directly comparable with a flor sherry produced in the traditional way. Much of the complexity of the true flor sherry is missing, but the product is valuable for blending. The method has been shown to be feasible for operation either in large or small scale wineries (Farafontoff 1964) and is used commercially.

With the submerged culture process, it is possible to obtain the basic flor character by using some of the non-film forming wine yeasts commonly used in winemaking. Thus it becomes possible to eliminate any risk of the flor film yeasts causing spoilage in table wines (Ough 1961). Russian workers have confirmed this finding. Castelli (1960) and Ivlev (1961) state that the general nature of the sherry character is not greatly affected by the yeast strain used, though there are differences in growth rates.

The submerged culture process described above is discontinuous, but continuous flow submerged culture systems have been described in Russia (Preobrajensky 1964, Martakov *et al* 1966). Recently Steinkraus (1972) has examined the continuous process from the point of view of obtaining the data necessary to design continuous culture plant. He concluded that such plant could be made stable in its operation and that continuous sherry production on a winery scale could readily be achieved.

Sensory data on the sherry produced by these newer methods are few, but Crowther and Truscott (1955) reported rapid ageing of the sherries they produced, and more recently Spanish workers (Cabezudo *et al* 1968) reported that 20 samples of sherries made in submerged culture were found by an expert taste panel to have characteristics comparable with aged *finos* made by the traditional process.

The aroma of flor sherry has been exhaustively studied by Webb and Kepner (1962, 1967), Webb *et al* (1964, 1967), Kepner and Webb (1968) and Galetto *et al* (1966). It had been proposed that the simple ratio of acetaldehyde to ethyl acetal gave a good index of sherry quality, but the more recent work shows that several acetals occur, all with intense characteristic odours. The major components of baked, flor and submerged culture sherries are rather similar, with the obvious difference of the high level of acetaldehyde in the sherries made in submerged culture. A more detailed comparison between flor and submerged culture sherries (Preobrajensky 1969) examined about 50 volatile compounds. The surface fermentation yielded high qualities of higher alcohols with a pleasant fruity smell as well as acetals and ethyl esters such as ethyl laurate. On the contrary the mixture of volatile materials in the submerged culture sherry was much less complex with the predominant volatile material being ethyl butyrate.

A mathematical analysis of the *solera* blending system has been made by Purser (1967) who recommends the best system for ageing and blending.

APPENDIX I

METHODS OF ANALYSIS

As the methods of analysis in the first edition listed below are part of the experimental procedures, they are reproduced in this edition without change. However, most of the methods have been supplanted by more recent techniques; Le Convention Internationale pour l'unification des méthodes d'analyses et d'appréciation des vins has an objective of standardisation and continuing improvement of the standard analytical methods. These recommended methods are published in the *Recueil des méthodes internationales d'analyse des vins*, Office International de la Vigne et du Vin, Paris, 1965.

ALCOHOL.

1. Pycnometer, A.O.A.C. official method.
2. Ebulliometer, using tables of Churchward and Johns (1940).
3. Dichromate oxidation, using the modification published by Joslyn and Amerine (1941), but distilling only 5 ml. of wine and making the distillate up to 50 mls. This method has proved very useful for following changes in alcohol during laboratory experiments.

pH. Glass electrode.

TOTAL TITRATABLE ACIDITY. Titration with 0.1N NaOH to phenol-red end point, using a spotting tile.

VOLATILE ACIDITY. A.O.A.C. official method with Sellier tube.

LACTIC ACID. Permanganate oxidation. (Ribéreau-Gayon and Peynaud 1937).

MALIC ACID. Permanganate oxidation (Peynaud 1938).

TARTARIC ACID. A.O.A.C. official method.

GLYCEROL. Periodate oxidation (Peynaud 1948).

REDUCING SUGAR. Hagedorn-Jensen method following clarification with zinc sulphate and sodium hydroxide.

FERMENTABLE SUGAR. Difference between total reducing sugar and the residual sugar determined after fermentation of the clarified solution with four per cent. of washed baker's yeast for 30 minutes at 37° C.

ALDEHYDES. The following adaption of the method of Jaulmes and Espezel (1935).

(a) *Total aldehyde.*

Solutions.

Neutral buffer. 3.35 gm. KH_2PO_4 and 15 gm. $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ per litre.

Bisulphite solution 0.945 gm. anhydrous Na_2SO_3 and 7.5 mls. 1.0 N H_2SO_4 per litre.

Starch indicator. 1 per cent. solution.

HCl. 250 ml. concentrated HCl per litre.

Borate buffer. 8.75 gm. boric acid and 400 ml. of 1.0 N NaOH per litre.

Phosphoric acid. 50 ml. of concentrated H_3PO_4 per litre.

Iodine solution 0.0227 N (1 ml. is equivalent to 0.50 mgrm. acetaldehyde).

METHOD. 5 (or 10) ml. of wine and an equal quantity of phosphoric acid solution are pipetted into a 100 ml. flask of an all-glass still and 20 ml. of water added. 5 ml. of the neutral buffer, 20 ml. of water and 1 ml. of the bisulphite solution are placed in the receiving flask (a 125 ml. erlenmeyer).

The distillation flask is connected to the still and about three-quarters of the liquid distilled over. During distillation the end of the condenser dips below the liquid in the receiving flask and the receiving flask stands in a beaker containing crushed ice.

When distillation is complete, the receiving flask is stoppered and left at room temperature for 15 minutes.

Then 3 drops of starch indicator and 1 ml. of HCl solution are added and the excess bisulphite titrated with iodine. Ten ml. of the borate buffer are then added and the liberated bisulphite at once titrated with iodine from a micro-burette. Each ml. of iodine used in the second titration is equivalent to 0.5 mgrm. of acetaldehyde in the distillate.

The determination of total aldehyde by distilling in the presence of phosphoric acid has been criticized by Ribèreau-Gayon and Peynaud (1947) on the grounds that products which react with bisulphite are formed from sugars during distillation in the presence of acids and that consequently one does not obtain a well defined and reproducible result. Although it is agreed that the method is unreliable when applied to sweet wines and perhaps with red wines, it has been found to give highly reproducible results when applied to flor sherries in this laboratory.

(b) *Free aldehyde*: As for total aldehyde but instead of being acidified with phosphoric acid, the wine was neutralized and buffered at about pH 8.5 with a borate buffer (25 grm. sodium borate and 25 ml. of 1.0 N H_2SO_4 per litre) before distillation.

ACETAL. Method of Peynaud and Maurie (1938).

NEUTRAL ESTERS. Method of Peynaud (1936).

VOLATILE ESTERS. 50 ml. of wine was distilled with 5 ml. of water till about 50 ml. of distillate had been collected. The receiving flask was surrounded by crushed ice during distillation. The distillate was neutralized to phenolphthaleine with 0.1 N NaOH and an excess of 15 ml. of NaOH added. This was then boiled under a reflux condenser for 30 minutes, cooled and titrated with 0.1 N HCl. From the titration figure and blank determinations the amount of NaOH required to saponify the esters was calculated.

TANNIN. A.O.A.C. official method.

SO_2 . Ripper method.

ACETYLMETHYLCARBINOL.

(a) *Qualitative*—Voges-Proskauer test using the modification of Vaughn, Mitchell and Levine (1939) and adding a small amount of 2 per cent. peptone solution to the wine.

(b) *Quantitative*—Method of Kniphorst and Kruisheer (1937).

DIHYDROXYACETONE. Method of Silberstein and Rappaport (1928).

CULTURE MEDIA

The following culture media have been used during the investigations.

1. DILUTED GRAPE JUICE

Grape juice, 300 mls.

Ammonium sulphate, 4.5 gm.

Dipotassium phosphate, 0.75 gm.

Magnesium sulphate, 0.10 gm.

Water, 700 mls.

Sterilization. Because of the low pH this medium can be effectively sterilized by heating to 100° C. in the steamer once for 20 minutes.

2. DILUTED GRAPE JUICE AGAR

As for medium No. 1 but 4 per cent. agar is added.

3. MALT EXTRACT

Commercial concentrated malt extract is diluted with water to a density of about 1.040 (usually about a 5 per cent. solution). The solution is autoclaved at 15 pounds for 20 minutes and filtered to remove substances coagulated by heat. It is then dispensed in tubes, flasks or bottles and sterilized in the steamer on two successive days.

4. MALT EXTRACT AGAR

As for medium No. 3, but 2 per cent. of agar is dissolved in the clarified liquid.

5. MALT EXTRACT GELATIN

As for medium No. 3, but 12 per cent. gelatin is dissolved in clarified liquid.

6. SPORULATION MEDIA

Gorodkowa's agar.

Vegetable extract agar of Mrak, Phaff and Douglas (1942).

Carrot wedges.

Potato wedges.

Dextrose-acetate agar of Adams (1949).

Gypsum slants (Graham and Hastings 1941).

7. BASAL MEDIA FOR TESTING NITROGEN UTILIZATION

Synthetic media of Stelling-Dekker (1931).

Nitrogen free medium of Wickerham (1946) supplied in powdered form by Difco Laboratories.

8. BASAL MEDIA FOR TESTING FERMENTATION OF SUGARS AND UTILIZATION OF ALCOHOL AND OTHER CARBON COMPOUNDS

Difco yeast extract—1 per cent. solution for fermentation tests.

Synthetic medium of Stelling-Dekker (1931) for alcohol utilization tests.

Synthetic medium of Wickerham and Burton (1948), supplied in powdered form by Difco Laboratories, for testing fermentation of sugars and utilization of various carbon compounds.

9. ARTIFICIAL MEDIUM FOR STUDY OF METABOLISM OF FLOR FILM

Difco yeast extract, 1 gm.

Ammonium sulphate, 0.5 gm.

Potassium dihydrogen phosphate, 1 gm.

Magnesium sulphate, 1 gm.

Calcium chloride, 0.5 gm.

Glycerol, 5 gm.

Ferric citrate 5 mls. of 0.6 per cent. solution—
about 6 p.p.m. of iron.

Water, 860 mls.

pH is adjusted to 3.2 with HCl and the solution sterilized by heating once with the steamer for 30 minutes. When cool, 140 mls. of ethyl alcohol is added with aseptic precautions and the solution dispensed in sterile flasks or bottles as required.

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